Food Contaminants:
Sources and Surveillance

Edited by
Colin S. Creaser and Rupert Purchase

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Preface

This book contains contributions based on the proceedings of two symposia on food contamination held in London in April 1989 and May 1990, both of which were organized jointly by the Environment, Food Chemistry, and Toxicology Groups of the Royal Society of Chemistry.

The aim of these meetings was to assess the extraneous chemical contamination of food from two sources: firstly, *food-chain contaminants* – the presence of plant toxicants or fungal metabolites in food, or the contamination of food from environmental sources (airborne, aquatic, and terrestrial); secondly, *food-production contaminants* – contaminants of man-made origin brought about by a desire to facilitate food production and distribution.

The surveillance of food contaminants through analytical and toxicological investigations has stimulated public awareness, and in turn has led to changes in the control of these substances by the legislators. Important as these issues are, they should be placed in the broader context of food hazards in general. A list of hazards associated with food drawn up by the FDA in the United States, and reiterated by the late Professor Leon Golberg, a pioneer of toxicology both in the UK and the USA (*Chem. Ind. (London)*, 1982, 354), is as follows (in decreasing order of importance): food-borne disease of microbial origin, malnutrition, environmental contaminants, toxic normal constituents of food, pesticide residues, and food additives. It is through the vigilance of the agriculture and food industries and legislative bodies that contamination problems of all these types are comparatively rare.

The contributions in this volume concentrate on the contamination of food by chemicals arising from environmental and food-production sources. Chapter 1 is concerned with food-chain contaminants present in food as natural components of the diet. This is followed by discussion of the chlorinated dioxins and furans (Chapter 2), and polycyclic aromatic hydrocarbons (Chapter 3). After an introduction to the control and surveillance of food-production contaminants (Chapter 4), four areas of activity are described: migration from food contact materials with particular reference to plastics (Chapters 5 and 6), the regulatory control and analysis of

veterinary products (Chapters 7 and 8 respectively), the analysis of pesticides in drinking water (Chapter 9), and finally the problem of food taints (Chapter 10).

We thank our colleagues from the RSC subject groups for their contribution to the organization of the two symposia which led to the publication of this book: Dr. David Henshall, Dr. Robert Massey, Dr. Martin Shepherd, and Dr. David Taylor.

Colin Creaser and Rupert Purchase
March 1991

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CHAPTER 1

Natural Toxicants in Food

JENNY A. LEWIS AND G. ROGER FENWICK

1 Introduction

The maintenance of the safety of the food supply is an essential function of the state. The food industry has a statutory obligation to provide safe, wholesome food for its customers but it is the duty of government to ensure that satisfactory methods and understanding exist to fulfil this obligation. As has become all too apparent in the UK recently, when agricultural products cannot be sold by virtue of the presence of recognized hazards, producers lose income, trade is damaged, and consumers are justifiably concerned. Public awareness of the importance of safe, highly nutritious food has never been higher, and consumers are actively seeking factual information and reassurance about the safety of their diets and of the individual foods they consume. Such concern and interest is to be welcomed, even at the expense of the sometimes ill-informed, and frequently emotional, arguments which can develop as a consequence.

Much public concern has been focused on chemicals in the food chain, be these agrochemicals, post-harvest dips or sprays, recognized and approved additives, environmental contaminants, or adulterants. There has, in comparison, been much less attention paid to potentially harmful chemicals which occur naturally in our diet as a consequence of their presence in plants used as foods and animal feeding stuffs. Such compounds, usually termed natural toxicants, are the subject of this chapter. Serious health problems due to natural toxicants are not new; indeed one such episode is described in the Book of Numbers, Ch. 11, vv. 31–33. Another, admittedly extreme, example from history was the periodic flare-up of ergotism. This occurred for over a thousand years and into the present century, claiming many hundreds of thousands of victims. It is now recognized that the causes of the epidemics were the naturally occurring alkaloids produced by the rye fungus, *Claviceps purpurea*, and it is interesting to note that the diminution of the problem was not a consequence of any public or domestic health measures, but rather a change in social and agricultural practice with the potato replacing rye as a staple of the European diet.

In general, however, the effects of natural toxicants are more likely to be chronic rather than acute and consequently, even when detected and measured, they are frequently impossible to relate to individual dietary components, let alone particular chemical constituents thereof. Food is probably the most chemically complex material normally encountered by the population at large. The number of naturally occurring compounds in food plants probably exceeds half a million, ranging from volatile flavour compounds to macromolecular proteins or polysaccharides. The preparation of food, involving a variety of processes from mixing to chopping, boiling, and frying, produces complex secondary chemical reactions, the nature and extent of which are only now becoming clear to the food chemist. Whilst many of these reactions are perceived by the senses as desirable, *e.g.* in the development of anticipated flavour, texture, and colour, this is not necessarily the case.

Despite the continuing development and refinement of techniques for the separation, isolation, and structural determination of individual chemical compounds, only a minority of those present in food have been identified and very many fewer have been subject to biological scrutiny at anything like the level carried out on agrochemicals, pollutants, food additives, and colorants. Various reasons may be suggested for this state of affairs, including the cost of pharmacological and toxicological testing, the frequent difficulties encountered in the isolation of these natural food components in amounts and purities sufficient for biological examination, and, perhaps most importantly, the overemphasis on the screening of those exogenous substances in food which are generally believed by the public to be the only 'chemicals' present and which have become a major focus of attention for the media and pressure groups. It is unfortunate that food scientists, and food chemists in particular, have so singularly failed to correct this misapprehension and to explain to the public that, in the context of food, the word *chemical* refers to both natural and man-made compounds and that, in contrast to what the advertizing companies would lead us to believe, 'natural' is not necessarily synonymous with 'wholesomeness' and 'safety'.

2 The Risk Due to Natural Toxicants

The six principal categories of food hazard are listed in rank order in Table 1. This ranking, based upon objective scientific criteria (including the severity, incidence, and onset of biological symptoms) was determined by Wodicka in 1971.[1] Around the same time Hall (1971)[2] pointed out that the ranking was not linear and that the hazard associated with environmental pollutants and natural toxicants was $\sim 10^3$-fold less than that originating from nutrient imbalance (which embraces both excess and deficiency). Furthermore, contrary to media comment and public concern, the risk due

[1] V. O. Wodicka, *Food Chem. News*, 1971, March 1st, 130.
[2] R. L. Hall, *Flavour Ind.*, 1971, August, 455.

Table 1 *Priority of food hazards (Wodicka, 1971)*

1 Microbial contaminants
2 Nutritional imbalance
3 Environmental contaminants
4 Natural toxicants
5 Pesticide residues
6 Food additives

to the presence of pesticide residues or food additives is considered to be $\sim 10^2$-fold lower than that resulting from natural toxicants.

In 1971, Hall[2] described subjective rankings obtained as a result of the comments of the press, food industry, 'fringe hysteria' (presumably the opposite of informed public opinion), and the US FDA regulatory authorities. The only common feature was that natural toxicants were ranked least important by all these groups. A recently constructed questionnaire, used by staff at the Institute of Food Research in 1988–89 (R. Shepherd and G. R. Fenwick, unpublished) confirms this finding and supports the comment of the 1985 UK Food Safety Research Consultative Committee that 'there is an almost complete mismatch between areas of known quantifiable hazard on the one hand and public concern and legislative activity on the other'.

One reason for this situation has been mentioned above, the general absence of readily identified, and acute, symptoms associated with natural toxicants. The terminology used may also be confusing; if natural, in the public mind, is indicative of goodness, freshness, and wholesomeness and encapsulates an ecologically desirable 'chemical-free', simple state of living, then toxicant is perceived as being related to food poisoning or linked to pollution, adulteration, and contamination of the food supply. Thus the term 'natural toxicant' offers an apparent contradiction, describing an area of hazard little understood or regarded by consumers and legislators alike.

Paradoxically, as inspection of any of a number of books on natural toxicants will confirm, there is no shortage of information available on the biological effects of chemicals isolated from plants, including those consumed by animals and man (for example Cheeke, 1989[3]). The problem is that the majority of such studies have used *in vitro* or other assays, the relevance of which to the human condition is open to question. There is no doubt that the techniques available to the chemist for analysing food components have generally far outstripped the complementary biological information which is necessary to put the analytical data into a scientific and social context. This in turn, makes assessment of research priorities difficult for the administrator. In the next sections the elements of a coherent natural toxicant research strategy are discussed and criteria for assessing priorities are suggested.

[3] 'Toxicants of Plant Origin', ed. P. R. Cheeke, Volumes I–IV, CRC Press Inc., Boca Raton, Florida, 1989.

3 Natural Toxicant Research

As part of an overall food safety policy, a research programme on natural toxicants should have the elements indicated in Figure 1. Following the identification and characterization of an effect, which might be acute or chronic, the causative principle(s) should be separated and identified. Methods of analysis should then be developed to enable calculation of exposure data on both the population at large and sub-groups judged to be particularly at-risk. It may also be necessary to develop additional methods of analysis to facilitate the clinical or toxicological studies which are also an integral part of this programme. It will be necessary to isolate sufficient of the active principle(s) to optimize and validate these analytical studies and also to facilitate the biological investigations. The aim of the latter should be to determine the potency of the identified compounds in man or in suitable animal models judged to have relevance to the human condition. Ideally toxicological investigations should include examination of the mechanism underlying the observed effect since this may enable extrapolation to chemical compounds having structural similarities. The study of selected compounds under conditions of long-term, low-level exposure (although, as will be seen later, exposures are not necessarily low) would, arguably, be of more benefit than wider screenings using tissue culture or micro-organisms.

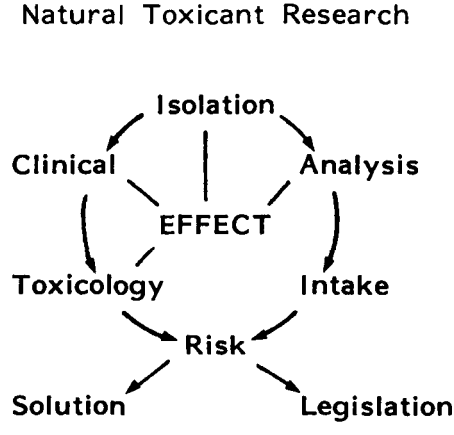


Figure 1 *The basic elements of a coherent programme on natural toxicants*

Any risk associated with consumption of a particular foodstuff containing identified natural toxicants will be determined by the exposure data, the metabolic activation or detoxification of the active principle(s), and the biological potency. Should such calculation result in the identification of a risk then the question of reducing or removing the risk has to be considered. Options for this will be considered below.

It may be predicted, with some confidence, that exposure to a variety of natural toxicants will increase during the present decade. There are various

reasons for this. First, as a result of nutritional advice and recommendations, consumption of green vegetables, plant protein, and fibre will increase. Although this advice is certainly correct it must be remembered that such dietary changes will be associated with increased exposure to a range of biologically active secondary metabolites whose long-term effects, for good or ill, on human health cannot yet be ascertained. Secondly, there are dietary fashions – for example, the consumption of potato peel referred to elsewhere. The increased reliance of some individuals on 'health' foods and herbal beverages and tonics may also be seen in this context. In most cases the botanical composition, and hence chemical content, is unknown. Exposures may currently be very large and continuous – very different from earlier times when the plants, their decoctions, and products were used infrequently against specific illnesses, or in response to particular symptoms. A third reason, amplified below, relates to changes in agricultural practice, with external (agrochemical) plant protection being replaced by 'natural' processes – often associated with the compounds considered here, in the context of food, as natural toxicants.

Finally it should be emphasized that attention should not be uncritically directed at toxic compounds in plants *per se*, because they may subsequently be rendered inactive by storage, processing, metabolism or digestion. Conversely inactive species may be activated by chemical transformations caused by the same processes.

4 Criteria for Assessing Research Priorities

Suggested criteria are listed in Table 2 and will be discusssed individually below.

Table 2 *Criteria for priority assessment*

Epidemiological relationships
Physiological/diet-related disturbances in man
Performance data on animals
Extent of use of wild types in plant breeding
Physicochemical/chemical effects in non-food systems
Serendipity

(i) Epidemiological relationships are the most difficult to identify, especially as diets become more and more internationalized. Examples generally relate to the dietary habits of particular groups of people in particular societies and include, for example, oesophageal and gastric cancers in Japan and South America related to bracken-derived carcinogens (Fenwick, 1988[4]), haemolytic syndromes associated with legume consumption in individuals of Mediterranean extraction (Chevion *et al.*, 1983[5]), and

[4] G. R. Fenwick, *J. Sci. Food Agric.*, 1988, **46**, 147.

[5] M. Chevion, J. Mager and G. Glaser, in 'Handbook of Naturally-occurring Food Toxicants', ed. M. Rechcigl, jr., CRC Press Inc, Boca Raton, Florida, 1983, pp. 63–79.

hypoglycaemia in Jamaica which has been shown to be due to consumption of the unripe fruit of the ackee tree (Kean, 1976[6]).

(ii) Diet-related disturbances in man, though infrequent, do enable studies to be focused on particular diets, and even foodstuffs, so that cause/effect relationships may often be readily identified. Thus, for example, the acute physiological disturbance which follows consumption of green or damaged potatoes (see below) or of squash or zucchini containing high levels of cucurbitacins (Rymal *et al.*, 1984[7]), severe clinical symptoms due to the consumption of certain crustacea and Pacific fish (Schantz, 1973[8]), and the problems of weakness which have been shown to relate to excessive consumption of liquorice confectionery containing the sweet saponin, glycyrrhizin (Lutomski, 1983[9]). It is not necessary for food plants to be consumed to cause problems; thus celery pickers are susceptible to severe dermatitis, the result of contact with photocarcinogenic psoralens, the accumulation of which in the plant may be increased as a consequence of damage, infection, or stress (Beier *et al.*, 1983[10]). Finally cassava (manioc) is a dietary staple in many parts of the world; nevertheless, if not properly processed before being consumed it may cause death or severe illness as a result of the presence of glycosides which yield hydrogen cyanide on hydrolysis. These compounds may also cause chronic effects including amblyopia, damage of the optic nerve, tropical atoxic neuropathy, and thyroid disorders (Conn, 1973[11]).

(iii) In some circumstances, performance data from animals may be useful, for example reproductive disturbances in farm and other animals grazing on pasture legumes or consuming soya-based rations which are known to contain isoflavone- and coumestan phytoestrogens (Price and Fenwick, 1985[12]), damage to liver and lungs in cattle fed sweet potatoes which is now known to be due to the presence of furanoterpenes (Wilson *et al.*, 1978[13]), and severe illness and death in animals exposed to a range of pyrrolizidine alkaloids occurring in pasture weeds (World Heath Organization, 1988[14]). In all these cases the same compounds may be regularly consumed by humans.

[6] E. A. Kean, 'Hypoglycin', Academic Press, New York, 1976.
[7] K. S. Rymal, O. L. Chambliss, M. D. Bond, and D. A. Smith, *J. Food Protect.*, 1984, **47**, 270.
[8] E. J. Schantz, in 'Toxicants Occurring Naturally in Foods', ed. F. M. Strong, National Academy of Sciences, Washington, DC, 1973, pp. 424–447.
[9] J. Lutomski, *Pharm. Unserer Zeit*, 1983, **12**, 49.
[10] R. C. Beier, G. W. Ivie, and E. H. Oertli, in 'Xenobiotics in Foods and Feeds', ed. J. W. Finley and D. E. Schwass, American Chemical Society, Washington, 1983, pp. 295–310.
[11] E. E. Conn, in 'Toxicants Occurring Naturally in Foods', ed. F. M. Strong, National Academy of Sciences, Washington, 1973, pp. 299–308.
[12] K. R. Price, and G. R. Fenwick, *J. Food Add. Contam.*, 1985, **2**, 73.
[13] B. J. Wilson, J. E. Garst, R. D. Linnabary, and A. R. Doster, in 'Effects of Poisonous Plants on Livestock', ed. R. F. Keeler, K. R. Van Kampen, and L. F. James, Academic Press, New York, 1978, pp. 311–333.
[14] World Health Organization, 'Pyrrolizidine Alkaloids, Environmental Health Criteria 80', World Health Organization, Geneva, 1988.

However, care has to be taken not to extrapolate uncritically from the results of animal, or *in vitro*, experiments to man. To cite but one example, severe haemolytic anaemia, possibly with fatal consequences, may result from the ingestion of cruciferous forages by cattle and other animals. The haemolytic precursor has been identified (Smith *et al.*, 1974[15]) as *S*-methylcysteine sulphoxide (SMCO), a compound also found in brassica vegetables, beans, and alliums. However, these foods pose no risk to human health since in man, unlike the above animals, SMCO is not broken down to the active haemolysin, dimethyl disulphide. Glucosinolates (formerly termed mustard oil glycosides) are present in animal feeding stuffs such as oil seed rape, and considerable effort and expenditure have been directed at reducing the exposure of farm animals to these compounds, or more correctly to the various products of their chemical/enzymic breakdown. Possible consequences of the presence of such compounds in the human diet will be considered later.

(iv) The effects of the use of wild-type plants (*i.e.* those which are not cultivated and frequently inedible) in breeding programmes to achieve primary objectives, such as increased yield or disease resistance, have not been widely studied. Since increased disease resistance is in many instances due to increased levels of secondary metabolites which exhibit wide ranging biological activities, there is reason to be vigilant in this respect. A well documented, but highly relevant, example of the unexpected and potentially hazardous consequences of plant breeding is that of the potato. The cultivar Lenapé, possessing excellent processing and agronomic characteristics, was being introduced into the USA two decades ago after having completed all the then-applicable screens and trials. The sudden realization that very high levels of glycoalkaloids possessing anticholinesterase-activity could be produced under particular combinations of day-length and temperature in the North Eastern states of the USA led to the rapid removal of this variety (Zitnak and Johnson, 1970[16]) and a public health problem of major proportions was narrowly averted.

Investigation subsequently showed the problem to be a consequence of the use in the breeding programme of the wild potato *Solanum chacoense*. A rather similar situation, not well publicized, later arose in the UK and involved the use of another wild potato, *S. vernii*, which was being used to introduce eel-worm resistance. Very late on in the breeding programme material was screened for glycoalkaloid content and, as a result of disturbingly high levels, many potentially commercially attractive clones had to be withdrawn.

(v) Saponins are found in beans and peas, and their effectiveness in reducing plasma cholesterol levels in animals and man (see Price *et al.* 1987[17]) has led to suggestions that saponin exposure should be increased as

[15] R. H. Smith, C. R. Earl, and N. A. Matheson, *Trans. Biochem. Soc.*, 1974, **2**, 101.
[16] A. Zitnak and G. R. Johnson, *Am. Potato J.*, 1970, **47**, 256.
[17] K. R. Price, I. T. Johnson, and G. R. Fenwick, *CRC Crit. Rev. Food Sci. Nutr.*, 1987, **26**, 27.

a means of naturally reducing the risk of coronary artery disease; such increased exposure might be achieved by increased consumption of legumes, developing new varieties with elevated saponin levels, or *via* regular intakes of saponin-containing health supplements. However, such suggestions have to be treated with caution, since it is well known from a variety of uses that saponins possess potent surface-active properties. Studies have demonstrated that saponins may exhibit a significant effect on the gut wall, thereby increasing its permeability to other dietary components or xenobiotics (Johnson *et al.*, 1986[18]; Gee *et al.*, 1989[19]). These effects are strongly structure-dependent, suggesting in turn that analytical information on levels and exposure of individual saponins is more important than data based upon 'total saponin' intake.

(vi) The final criterion, serendipity, rarely receives due credit; in this regard natural toxicant research is no different from many other branches of scientific investigation. A good example is the finding of substantially increased levels of oestrogenic activity in human urine following microbial conversion of natural isoflavone glycosides (such as those in soya) to more active metabolites (Axelson *et al.*, 1984[20]). This observation stemmed from the routine screening of patients rather than from any overt examination of oestrogenic effects.

5 Natural Toxicants

5.1 Glycoalkaloids

Potatoes and other members of the Solanaceae contain toxic glycoalkaloids, mainly α-chaconine and α-solanine (Figure 2), which interfere with the normal function of the central nervous system and also irritate the gastro-intestinal tract. These compounds are present in highest quantities in the above ground parts (flowers, $3–5 \times 10^3$ p.p.m.; leaves 400–1000 p.p.m.) and in the sprouts ($2–4 \times 10^3$ p.p.m.). Levels in the tuber vary, and are found mainly in the skin and peel region (150–600 p.p.m.); the significance of this from the food preparation and processing standpoint is that peeling reduces the glycoalkaloid content to 10–50 p.p.m.

A variety of factors, including genetic origin, adverse post-harvest handling and storage, environment and location, physiological stress, and processing lead to the increased synthesis, and accumulation, of glycoalkaloids in the tuber (Jadhav *et al.*, 1981[21]). Acute illness, and even death, in animals and man have followed the consumption of damaged, rotten,

[18] I. T. Johnson, J. M. Gee, K. Price, C. L. Curl, and G. R. Fenwick, *J. Nutr.*, 1986, **116**, 2270.

[19] J. M. Gee, K. R. Price, C. L. Ridout, I. T. Johnson, and G. R. Fenwick, *Toxicol. In Vitro*, 1989, **3**, 85.

[20] M. Axelson, J. Sjövall, B. E. Gustafsson, and K. D. R. Setchell, *J. Endocrinol.*, 1984, **102**, 49.

[21] S. J. Jadhav, R. P. Sharma, and D. K. Salunkhe, *CRC Crit. Rev. Toxicol.*, 1981, **11**, 21.

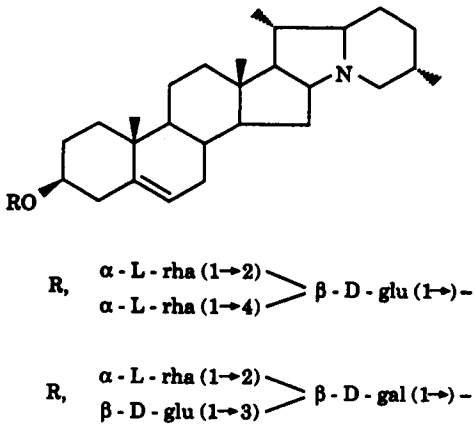


Figure 2 *Structures of the potato glycoalkaloids, α-chaconine (upper) and α-solanine (lower)*

green, sprouted, or blighted potatoes – *i.e.* low quality, sub-standard produce. Both α-chaconine and α-solanine are potent cholinesterase inhibitors and symptoms associated with such pharmacological activity include drowsiness, difficult or laboured breathing, weakness, paralysis, and lack of consciousness. Their effects on the gastrointestinal tract lead to inflammation of the intestinal mucosa, haemorrhage or ulceration, abdominal pain, and diarrhoea.

The most recent outbreak of potato poisoning in the UK occurred ten years ago in Lewisham. Seventy-eight schoolboys were taken ill 7–19 hours after a meal (containing potatoes which were later found to contain levels of glycoalkaloids in excess of 300 p.p.m.); seventeen children required hospital treatment with three being described as seriously ill. Symptoms included sickness, headache, vomiting, diarrhoea, and abdominal pain (McMillan and Thompson, 1979[22]).

As a result of problems with Lenapé (see above), and other varieties, developed via the use of glycoalkaloid-rich wild type *Solanum* species, new methodologies were developed for the analysis of these compounds (see Coxon, 1984[23]). These proved inappropriate for the large-scale screening of breeding programmes and a simple, much more rapid, ELISA procedure was introduced (Morgan *et al.*, 1983[24]). Unlike food additives, there is no statutory control of glycoalkaloid levels in potatoes, but a generally recognized limit of 200 p.p.m. has been set – this being derived

[22] M. McMillan and J. G. Thompson, *Q. J. Med.*, 1979, **48**, 227.
[23] D. T. Coxon, *Am. Potato J.*, 1984, **61**, 169.
[24] M. R. A. Morgan, R. McNerney, J. A. Matthew, D. T. Coxon, and H. W.-S. Chan, *J. Sci. Food Agric.*, 1983, **34**, 593.

from the finding (Sinden *et al.*, 1976[25]) that above this level the potato becomes increasingly bitter and toxic. Most tubers from table varieties of potato are well below this limit, and the likely human exposure is further reduced if the peel is removed; thus calculations of the UK mean daily intakes have produced figures of 5–31 mg (Ridout *et al.*, 1988[26]). It is worth while emphasizing the dramatic contrast with the approach to food additives in processed food. If it were to be seriously suggested that compounds with the biological activity of glycoalkaloids should be added to any food, let alone such a staple one as potatoes, there would be considerable, and justified, concern.

Amongst certain individuals the exposure to glycoalkaloids may be much larger, perhaps four-fold or greater. This is a result of a current trend towards the consumption of potato peel which is considered, mistakenly, by some people to be a good source of dietary fibre and vitamins. The effects, *chronic* rather than acute, of regular consumption of the peel (from jacket potatoes, snack products made wholly from peel, or potato skins) are largely unknown; however recent studies (Gee *et al.*, 1989[19]) have demonstrated glycoalkaloids to possess potent gut-permeabilizing activity, and further work is in progress to examine the possible clinical consequences which may ensue if this results in the passage across the gut of other dietary components, for example those able to provoke the immune system.

5.2 Glucosinolates

Consumption of leafy and root brassica vegetables in the UK is greater than in other West European countries and North America. There has been a trend in recent years away from cabbage and Brussels sprouts, towards the more delicately-flavoured cauliflower, calabrese, and Chinese cabbage. All these vegetables contain glucosinolates, a complex class of sulphur-containing glycosides. Under the influence of an enzyme, myrosinase, also found in the plant, glucosinolates are hydrolysed to yield an unstable aglucone which subsequently decomposes to provide a range of products (dependent on R, Figure 3) The chemical nature and properties and the biological effects and potencies of these compounds are determined by the nature and amount of the parent glucosinolates and by the conditions of the breakdown (Heaney and Fenwick, 1988[27]).

A great deal of human and financial resource has been expended in many countries over the past two decades with the aim of reducing the intakes of glucosinolates in farm animals, the intake being a consequence of the use of rapeseed products in livestock rations. As a result new

[25] S. L. Sinden, K. L. Deahl, and B. B. Aulenbach, *J. Food Sci.*, 1976, **41**, 520.
[26] C. L. Ridout, S. G. Wharf, K. R. Price, I. T. Johnson, and G. R. Fenwick, *Food Sci. Nutr.*, 1988, **42F**, 111.
[27] R. K. Heaney and G. R. Fenwick, in 'Natural Toxicants in Food, Progress and Prospects', ed. D. H. Watson, Ellis Horwood, Chichester, 1988, pp. 76–109.

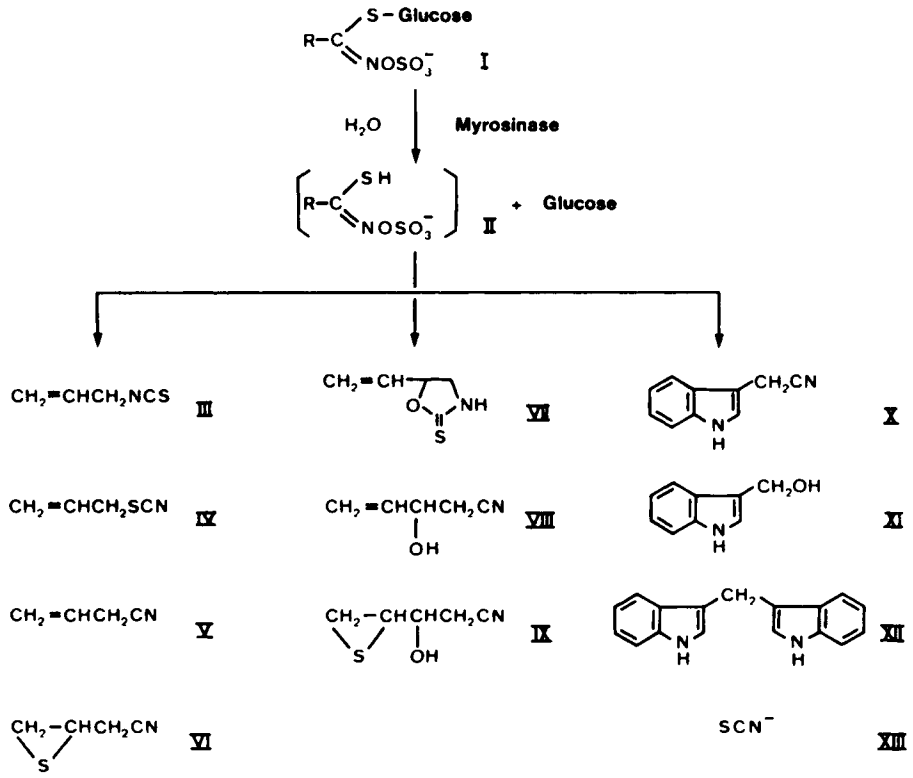


Figure 3 *Products of myrosinase-induced hydrolysis of glucosinolates*

'low-glucosinolate' varieties have been developed and now dominate the market place. The European Commission has assisted in this process by offering a premium to growers of such 'improved' varieties and has expressed the intention to remove all financial support from varieties having a higher glucosinolate content from 1991. The main problems associated with the presence of glucosinolates in animal rations are poor growth and performance, liver damage, impairment of normal thyroid function, and interference with normal metabolic processes. Such problems have been described in detail by Mawson *et al.* (1989).[28]

In comparison, considerably less attention has been devoted to the effect of glucosinolates and their products in man. Thus although many studies in animals and *in vitro* systems have shown isothiocyanates (III, Figure 3) and 5-vinyloxazolidine-2-thione (VII) to inhibit normal enzymatic processes, nitriles (VIII) to be hepatotoxic, epithionitriles (VI, IX) to possess weak mutagenic activity, and both 5-vinyloxazolidine-2-thione and thiocyanate ion (XIII) to interfere with thyroid function, the effects of such compounds

[28] R. Mawson, R. K. Heaney and G. R. Fenwick, in 'Natural Toxicants – Volume II, Glycosides', ed. P. R. Cheeke, CRC Press Inc., Boca Raton, Florida, 1989, pp. 1–41.

in man are generally unknown (Heaney and Fenwick, 1988[27]; Mawson *et al.*, 1989[28]). One area where researches have been conducted is that of the goitrogenic properties of the latter compounds. The thione inhibits thyroid function by interfering with the formation and secretion of thyroxine. In animal studies this results in reduced growth rate and hyperplasia and hypertrophy of the thyroid. The effect of thiocyanate ion (which, unlike 5-vinyloxazolidine-2-thione, interferes with the binding of iodine), is overcome by adding extra iodine to the diet, and there has been speculation that dietary goitrogens might have a role to play in the aetiology of human thyroid disorders. However, studies have shown thiocyanate ion at normal dietary levels (Dahlberg *et al.*, 1984[29]), and Brussels sprouts rich in the glucosinolate precursor of 5-vinyloxazolidine-2-thione (McMillan *et al.*, 1986[30]) to have no adverse effect on circulating thyroid hormones. It is possible, however, that in regions of iodine deficiency, a brassica-rich diet may aggravate existing symptoms of thyroid dysfunction.

The mean daily intake of glucosinolates of 5% of the UK population exceeds 300 mg, approximately one third of this figure representing the intake of indole glucosinolates (Sones *et al.*, 1984[31]). These latter compounds, particularly rich in varieties of Brussel sprouts and Savoy cabbage (see Heaney and Fenwick, 1988[27]), have attracted attention in recent years for two reasons. First, these compounds, or more correctly their hydrolysis products (X–XII, Figure 3) have been shown in studies with experimental animals, and in man, to induce a wide range of enzymes involved in general detoxification processes of the body (McDanell *et al.*, 1988[32]), and also to have a very significant effect on reducing the numbers of tumours caused by a range of carcinogens, including 3,4-benzpyrene, 1,2-dimethylhydrazine, and aflatoxin B_1. This has led to the suggestion that such indoles have a part to play in the protection which brassica vegetables offer against human gastric and other cancers. The second property of these indoles is their ability to react chemically with nitrite to form non-volatile nitrosamines, known to be mutagenic and considered to act as carcinogens in animals and man (Wakabayashi *et al.*, 1985[33]). Studies are in progress to determine the relevance of such findings to the human condition and it is possible that future epidemiological investigations will examine in more detail than hitherto the possibility of interactions between nitrate, the precursor of nitrite, and nitrosatable indoles, such as are present in brassicas (and also some legumes).

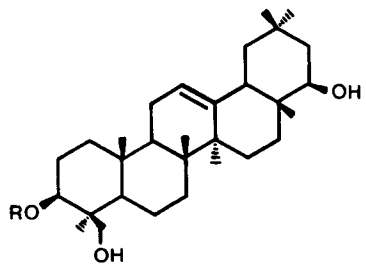
These findings clearly indicate that, depending upon one's perspective, natural compounds in the diet may be beneficial and deleterious; what is

[29] P. A. Dahlberg, A. Bergmark, L. Björck, A. Bruce, L. Hambraeus and O. Claesson, *Am. J. Clin. Nutr.*, 1984, **39**, 416.
[30] M. McMillan, E. A. Spinks, and G. R. Fenwick, *Human Toxicol.*, 1986, **5**, 15.
[31] K. Sones, R. K. Heaney, and G. R. Fenwick, *J. Sci. Food Agric.*, 1984, **35**, 712.
[32] R. McDanell, A. E. M. McLean, A. B. Hanley, R. K. Heaney and G. R. Fenwick *Food Chem. Toxicol.*, 1988, **26**, 59.
[33] K. Wakabayashi, M. Nagao, T. Tahira, H. Saito, M. Katayama, S. Marumo, and T. Sugimura, *Proc. Jpn. Acad.*, 1985, **61B**, 190.

thus needed is to gain as wide a knowledge of the biological effects as possible so that these may be included in any risk assessment or dietary benefit analysis. In many cases it is likely to be the *overall balance* of biological activity which will be important. This is also exemplified by the saponins, considered above and briefly below.

5.3 Saponins

The intestine-permeabilizing effects of many saponins, noted above, raises the question of interactions between different classes of antinutrients or toxicants. Thus saponins are found amongst widely eaten legumes (Price *et al.*, 1988[34]), exactly those foods which society is being urged to consume because of their high fibre and protein contents. These legumes contain a wide range of additional toxicants, including tannins, oligosaccharides, phytate, lectins, enzyme inhibitors, and oestrogenic isoflavones; the effect of saponins may thus be to increase the absorption of such compounds and thereby increase their biological activity. Some evidence in support of synergistic mechanisms may be found in the work of Alvarez and Torres-Pineda (1982),[35] who observed the gut permeability of soyasaponins (Figure 4) and lectins in an *in vitro* rabbit mucosal system to be greatly enhanced in combination. A similar finding has been recently reported for the glycoalkaloids α-chaconine and α-solanine (Gee *et al.*, 1989[19]). If such effects do occur in man, and it has to be emphasized that clinical investigations have yet to be completed, then particular consideration needs to be taken of minority groups, such as vegetarians, whose intake of legumes and associated toxicants is considerably greater than that of the population at large (Ridout *et al.*, 1988[26]).



R = rha(1→2)gal(1→2)glu A(1→)-

R = rha(1→2)ara(1→2)glu A(1→)-

R = gal(1→2)glu A(1→)-

R = ara(1→2)glu A(1→)-

Figure 4 *Structures of typical saponins from soya*

[34] K. R. Price, J. Lewis, G. M. Wyatt, and G. R. Fenwick, *Nahrung*, 1988, **32**, 609.
[35] J. R. Alvarez and R. Torres-Pineda, *Pediatr. Res.*, 1982, **16**, 728.

The presence in soya, and its products particularly, of large amounts of isoflavones and coumestans having obvious oestrogenic effects (Price and Fenwick, 1985[12]) may also be significant in this respect. Preliminary studies (Jones *et al.*, 1989[36]) have shown that whereas the population at large has a mean daily intake of such compounds below 1 mg, that of vegetarians (who currently number some 13% of women aged between 16 and 23) may be much higher (100 mg or more). Given the findings of Axelson *et al.*, (1984)[20] and the more recent work of Setchell and co-workers, which demonstrates that such compounds can have significant effects on mammalian reproductive physiology (Setchell *et al.*, 1987[37]), preliminary clinical or epidemiological investigations of subjects consuming large amounts of soya products would appear prudent.

5.4 Illudane Sesquiterpenes

It may come as something of a surprise to learn that bracken, that most natural of countryside plants, is highly toxic (Fenwick, 1988[4]). Numerous animal studies have demonstrated chronic and acute symptoms and there is persuasive epidemiological evidence of a link between gastric and oesophageal cancers in Japan (where young bracken fronds are considered a particular delicacy) and in regions of Central and South America (where the vector is considered to be the consumption by rural populations of milk from goats and cattle foraging on the plant). There have been extensive studies over the past forty years into the biological effects of bracken and in some cases contradictory results have been obtained. It now appears that the major toxic principle, in the plant itself, is the carcinogen, ptaquiloside (Figure 5), the levels of which may be greatly affected by geographical, seasonal, environmental and genetic variables.

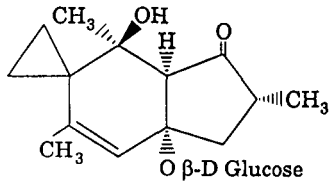


Figure 5 *Structure of the bracken carcinogen, ptaquiloside*

It is significant that in bracken research the knowledge of biological and toxicological effects (see Figure 1) is greatly in excess of the analytical information available, this being rather unusual in natural toxicant research. This lack of both a reliable analytical method and the resulting compositional data made it difficult if not impossible to compare the results

[36] A. E. Jones, K. R. Price and G. R. Fenwick, *J. Sci. Food Agric.*, 1989, **46**, 357.
[37] K. D. R. Setchell, S. J. Gosselin, M. B. Welsh, J. O. Johnston, W. F. Balistreri, L. W. Kramer, B. L. Dresser, and M. J. Tarr, *Gastroenterology*, 1987, **93**, 225.

of different laboratories. It is probable that, had a chemical assay or screening method been developed earlier, many of the problems still confronting researchers in this area would have been, at least partly, resolved.

There should be little cause for concern in the UK over the transfer of bracken carcinogens into milk and dairy products if these are obtained from cattle and processed centrally. First, the economics of conventional dairy farming are such that animals are raised on highly cultivated pasture where bracken is discouraged. Secondly, except under adverse environmental conditions (*e.g.* severe drought), cattle do not usually consume bracken in preference to other plants (although there are reports of cattle consuming bracken fronds, perhaps as a roughage, when the usual pasture is excessively lush). Once again there may be a risk if isolated communities, especially those espousing a simple, 'back to nature' philosophy, consume milk from individual cows or small numbers of cattle when feeding is not subject to control and if the milk is not bulk-processed and heat-treated. The raising of goats and the sale of goat's milk and products have increased substantially in recent years. Thus there are now more than 100 000 goats in the UK, the number in registered holdings having doubled between 1975 and 1983 (MacCarthy, 1988[38]). Such animals are usually kept on marginal land where bracken can occur. As there seem to be no reports about the effect of bracken on goats and the extent, if any, to which bracken carcinogens may be transferred to the milk, such studies would be very valuable.

Although the consumption of bracken fronds would not seem to pose a threat to human health in the UK, the recent finding by I. A. Evans and co-workers[39] that bracken spores are carcinogenic requires further study. The suggestion that farm-workers, foresters, and others regularly venturing into large areas of bracken during the period of sporulation should be encouraged to wear face masks does not seem an over-reaction.

6 Options for the Control of Natural Toxicants

The main options for the reduction of the hazard associated with the presence of natural toxicants in food and feeding stuffs are discussed in this section.

6.1 Elimination of Foods Containing Such Toxicants from the Diet

Providing that animals are fed under well defined, controlled, conditions the elimination of toxic ingredients from the ration should be relatively

[38] D. MacCarthy, 'Prodfact – 1988; A Comprehensive Guide to British Agricultural and Horticultural Produce', British Farm Produce Council, 1988, pp. 212–217.

[39] I. A. Evans, in 'Bracken, Ecology, Land Use and Control Technology', ed. R. T. Smith and J. A. Taylor, Parthenon Publishing Group, Carnforth, Lancs., 1986, pp. 139–146.

straightforward. Thus the apparently unrelated problems of egg taint and liver haemorrhage in laying hens have been solved in the UK by removing rapeseed meal from commercial layer rations (Fenwick and Curtis, 1980[40]). Where animals are free to forage then dietary control is much less easy, and good range or pasture management is essential.

The elimination of certain materials from the human diet poses considerably more of a problem, even for countries possessing centralized food production and distribution systems. State intervention can be effective, but only when supplemented by programmes of public education and when appropriate alternative foodstuffs are freely available. The lima bean contains cyanogenic glycosides, compounds which yield hydrogen cyanide upon hydrolysis. Levels of these glycosides in certain varieties are equivalent to, or greater than, 2×10^3 p.p.m. HCN, and, given the toxicity of this product, certain countries have legislated against the importation of beans containing levels greater than 200 p.p.m. (Conn, 1973[11]).

Decisions to avoid individual foods may also be made at the personal level. Thus certain people of Mediterranean extraction avoid legumes containing vicine or convicine since consumption leads to the onset of a haemolytic syndrome, favism (Chevion *et al.*, 1983[5]); people, having experienced migraine-like symptoms, avoid items such as bananas, cheese, and chocolate, which may contain the causative agents (Lovenberg, 1973[41]) and individuals prone to flatulence avoid foods which they find produce intestine gas (Price *et al.*, 1988[34]). These are all, of course, examples both of the varying composition of food products and also of the heterogeneity of the human population.

6.2 Exploitation of Normally Occurring Protective Factors in the Diet

The consumption of foods rich in dietary fibre has been suggested to offer protection against a wide range of Western diseases, including diverticular disease, colon cancer, diabetes, and heart disease (Trowell and Burkitt, 1981[42]). Much research has been conducted subsequently into understanding the mechanisms of these protective effects and into identifying sources of fibre particularly suited to alleviating individual ailments. Naturally-occurring phenolics exhibit antimutagenic and anticarcinogenic activity (Stich and Rosin, 1981[43]), onion and garlic oils are proven to be active against ischaemic heart disease and gastric cancer (Fenwick and Hanley,

[40] G. R. Fenwick and R. F. Curtis, *J. Sci. Food Agric.*, 1980, **31**, 515.
[41] W. Lovenberg, in 'Toxicants Occurring Naturally in Foods', ed. F. M. Strong, National Academy of Sciences, Washington, DC, 1973, pp. 170–188.
[42] H. C. Trowell, and D. P. Burkitt, in 'Western Diseases: Their Emergence and Prevention', ed. H. C. Trowell, and D. P. Burkitt, Edward Arnold, London, 1981, pp. 436–443.
[43] H. F. Stich, and M. P. Rosin, in 'Nutritional and Toxicological Aspects of Food Safety', ed. M. Friedman, Plenum Press, New York, 1981, pp. 1–29.

1985[44]; You *et al.*, 1988[45]), and certain glucosinolates, rich in the UK diet, are currently being examined for their anticarcinogenic and detoxifying properties (McDanell *et al.*, 1987[46]).

In general, however, although these studies are attracting increasing interest, amongst both scientists and the general public, considerable efforts will be needed before the effects in man are clinically proven, the possibility of undesirable side-effects is excluded, and dietary recommendations are proposed.

6.3 Development of Processes for Removing or Reducing Toxicants

Man has been adept at detoxifying food for many millenia. In addition to generating desirable flavour and texture, cooking is an effective detoxification process. The very first processes may well have developed from natural circumstances or accidents, such as fermentations or soaking. The relation between toxicity and bitter taste also served early man, the tongue functioning both as a bioassay and as an instrument of quality control. Many species of yam, cucurbit, cassava, and bean are inedible (due to excessive bitterness and toxicity) without prior processing. Quinoa, a protein-rich material, is potentially of considerable value to indigenous populations of South America, but the presence of toxic saponins means that prior processing is obligatory (Risi and Galwey, 1984[47]). Bitter varieties of cassava (manioc) are highly toxic because of the presence of cyanogenic glycosides and many deaths have been reported. Local techniques have been developed for processing cassava, including fermentation, steeping, and wet pounding, before consumption (Brouk, 1975[48]). It is vital that such detoxification processes take account of the local conditions and available, rural technology rather than unrealistic state-of-the-art 'first world' methodology.

An understanding of the origin, nature, and mode of action of natural toxicants may also facilitate their control. A knowledge that certain chemicals are accumulated as a response to microbial, insect, chemical, or mechanical damage will, hopefully, facilitate the establishment of agronomic, harvesting, and storage conditions designed to minimize such damage. A knowledge of the structures of individual toxicants, and of their chemical reactivities, may serve to suggest site-specific means of detoxification. Thus ammonia has been used to reduce the level of glucosinolates in

[44] G. R. Fenwick and A. B. Hanley, *CRC Crit. Rev. Food Sci. Nutr.*, 1985, **23**, 1.
[45] W. C. You, W. J. Blot, Y. S. Chang, Z. A. G. Ershow, Z. T. Yang, Q. An, B. Henderson, G. W. Xu, J. F. Fraumeni, and T. G. Wang, *Cancer Res.*, 1988, **48**, 3518.
[46] R. McDanell, A. E. M. McLean, A. B. Hanley, R. K. Heaney, and G. R. Fenwick, *Food Chem. Toxicol.*, 1987, **25**, 363.
[47] J. C. Risi and N. W. Galwey, *Adv. Appl. Biol.*, 1984, **10**, 145.
[48] B. Brouk, 'Plants Consumed by Man' Academic Press, London, 1975, pp. 96–97.

oilseed meals (Keith and Bell, 1982[49]) and ammonia and sulphur dioxide have been used to reduce the level of aflatoxins in peanut and corn products (Brekke *et al.*, 1979[50]). Care must be taken to ensure that the detoxified materials are not nutritionally inferior to the original product, especially when heat or extraction procedures are involved. Another factor, sometimes overlooked, is that the removal of one, known, toxicant may result in the formation of other, possibly unknown, biologically active species. Thus the detoxification of rapeseed or crambe meals with ferrous sulphate removes glucosinolates but results in the formation of significant amounts of nitriles, the toxicities of which have been well established (Heaney and Fenwick, 1988[27]; Mawson *et al.*, 1989[28]).

Although processing is clearly important, care must be taken to ensure that the introduction of new technology or even minor modifications to the processing conditions does not adversely affect the detoxification efficiency. Changes in culinary practice in the home may also have unforeseen consequences. Although it has long been known that beans contain toxic haemagglutins (lectins), the effects of these in human beings are minimal because of the heat treatments which precede consumption. There has recently been a trend, amongst certain sections of the population in the UK and elsewhere, to include partially cooked beans (especially red kidney beans) in salads and, since the haemagglutin has not been inactivated, a number of cases of 'food poisoning' have resulted (Bender and Reaidi, 1982[51]).

6.4 Introduction of New Plant Cultivars Containing Reduced Levels of Toxicants

Examination of the fresh produce currently available at retail outlets gives an indication of the manifold successes of the plant breeder. Improvements in yield, agronomic and harvesting characteristics, disease resistance, and tolerance to unfavourable conditions are all major objectives. In comparison much less emphasis has been placed upon improvement in food safety. This is partly due to the lack of pressure from legislators, consumer organizations, and industry. Where such pressures have been applied, *e.g.* over potato glycoalkaloids and rapeseed glucosinolates, breeders have responded. Since in many cases the presence of these biologically active species appears to be linked to plant resistance or defence, their mere removal does not necessarily produce an agronomically and commercially acceptable product and alternative protection factors may need to be introduced. The availability of a rapid, simple, and specific method for the

[49] M. O. Keith and J. M. Bell *Can. J. Anim. Sci.*, 1982, **62**, 547.

[50] O. L. Brekke, A. J. Peplinski, G. W. Nofsinger, H. F. Conway, A. C. Stringfellow, R. R. Montgomery, R. W. Silman, V. E. Johns and E. B. Bagley, *Trans. A.S.A.E.*, 1979, **22**, 425.

[51] E. A. Bender, and G. B. Reaidi, *J. Plant Foods*, 1982, **41**, 15.

screening of many thousands of plants, ideally in the early stages of growth, is a prerequisite for an effective breeding programme.

Consumer concern and attitudes towards the use of synthetic agrochemicals in intensive farming systems have resulted in a limited, but significant, move toward reduced-input farming systems. In order to grow crops successfully under wholly organic conditions, or in systems utilizing reduced agrochemical inputs, breeders and pathologists are actively seeking new plant varieties exhibiting improved 'natural' resistance. Such resistance may be associated with the presence of secondary metabolites and/or the ability effectively to synthesize and accumulate biologically active phytoalexins in response to physiological stress or damage. It is important to consider the possible consequences of such selection or plant compositional manipulation on human health and wellbeing, especially in the long term. For this reason it is vital that 'improved' varieties are not only screened for agronomic or processing characteristics, but that due consideration is also paid to biological activity (Ames, 1989[52]), difficult though this may be.

7 Conclusion

Contrary to public perceptions, artificial substances in the diet are no more hazardous than those occurring naturally and may be significantly less so. At present there have been relatively few rigorous comparisons of artificial and natural compounds, one reason for which being the absence of appropriate, simple, and inexpensive methodologies. In identifying the difficulties inherent in such work and in food toxicology generally it is important that these are not overstated and raised to the level of impossibilities. It is reasonable that equivalent attention be paid to natural toxicants, and their metabolites, as is currently given to additives, contaminants, and pesticides. The biological activity of the compound under investigation should be the major criterion, not whether it is natural or synthetic. Decisions on priorities must necessarily take account of local and national eating habits, intake, indicators of dietary trends or fashions, actual or potential toxicity, and developments in processing technology and 'novel' food materials.

In a perceptive article, Doull (1981)[53] has emphasized the importance of public education in food safety. Too much of the public's current 'education' is dependant upon alarmists, attention-seekers, and those with a vested interest in manufacturing and selling 'health foods' (which are commonly worthless and occasionally dangerous). For public acceptance and success, Doull concludes, any new food safety policy, embracing the consideration of natural toxicants, must encompass a mechanism for explaining, simply but concisely, the basis of food safety to consumers.

[52] B. N. Ames, in 'Important Advances in Oncology', J. B. Lippincott Co., Philadelphia, 1989.

[53] J. Doull in 'Food Safety', ed. H. R. Roberts, Wiley Interscience, New York, 1981, pp. 295–316.

In any event there may be pressing socioeconomic reasons which will limit the effectiveness of the programme. It is the poorest sections of society who are unable to afford the luxury of selecting their diet, of choosing high quality produce, and of discarding damaged items before cooking; in such cases problems of natural toxicants are probably greatest because the ingestion of toxicants is highest and because the toxic effects aggravate existing symptoms of malnourishment. For such people, the cutting away of damaged or infected areas of the potato represents not a minimizing of hazard but a loss of food. Until the underlying socioeconomic ills are dealt with, problems of natural toxicants exacerbated by ill health and starvation will remain.

As part of an overall programme of public health protection, natural toxicant research has an international dimension, benefitting from the exchange of ideas and data, technological and scientific collaboration, and commissioning of data bases. Public education, rather than scientific dogma, is a crucial factor in the implementation of an effective food safety programme.

Acknowledgements

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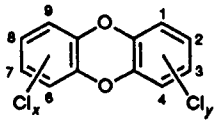
CHAPTER 2

Polychlorinated Dibenzo-p-dioxins, Polychlorinated Dibenzofurans, and the Food Chain

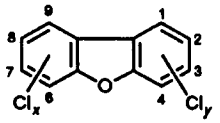
JAMES R. STARTIN

1 Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) (1) and polychlorinated dibenzofurans (PCDFs) (2) are environmental contaminants which are lipophilic, chemically stable, of low volatility, and which are known to be present, even if only at very low concentrations, in the fatty tissues of animals and humans. The high toxicity which some of these compounds exhibit in animal tests has resulted in concern about the possible health consequences of this ubiquitous exposure.



(1)



(2)

For some time the term 'dioxin' was associated solely with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) but this is only one of a large number of PCDD and PCDF congeners. Since both PCDDs and PCDFs can have from one to eight chlorine substituents there is much scope for positional isomers, as shown in Table 1, and the total number of PCDD and PCDF congeners is, respectively, 75 and 135. The toxicity of different congeners varies enormously and it is unfortunate that much of the public debate which has taken place in recent years has been concerned with 'dioxins and furans', or just with 'dioxins' as a generic term for both

Table 1 *The total number of isomers in each PCDD and PCDF homologue and the number with lateral substitution (with substitution at each of the 2-, 3-, 7-, and 8-positions)*

Number of chlorine substituents	*Number of isomers*			
	PCDD		*PCDF*	
	Total	*Laterally substituted*	*Total*	*Laterally substituted*
1	2		4	
2	10		16	
3	14		28	
4	22	1	38	1
5	14	1	28	2
6	10	3	16	4
7	2	1	4	2
8	1	1	1	1
Total	75	7	135	10

groups, without regard to the particular congeners concerned.

The beginnings of concern about the possible human health effects of PCDDs can be traced to a 1970 publication about the teratogenicity of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) where a footnote added in proof attributed the observed effects to the presence of 2,3,7,8-TCDD as a contaminant.[1] Subsequently other animal tests also revealed high acute oral toxicity and carcinogenicity. Public concern and scientific activity were increased by the realization that 2,3,7,8-TCDD had been present at significant concentrations in the 'Agent Orange' defoliant used by US forces in Vietnam, and by incidents such as the use of dioxin contaminated oils for dust control in horse arenas in Missouri and in the town of Times Beach. The explosion at Seveso, Italy in 1976 at a 2,4,5-trichlorophenol production facility led to the release of an estimated 2–3 kg of 2,3,7,8-TCDD and contributed substantially to concern and scientific effort, but had been preceded by a number of other, less well publicized, industrial accidents in other locations.

During measurement of the concentrations of TCDD present in tissue from people thought to have been exposed in such incidents, it was discovered that even control samples often contained measurable concentrations of 2,3,7,8-TCDD and other PCDDs and PCDFs, and these compounds are now recognized as being ubiquitous contaminants of the environment and of human tissues. Inhalation and direct contact can only account for a very small proportion of the typical human body burden, and the major part is thought to derive from intakes associated with food.

[1] K. D. Courtney, D. E. Gaylor, M. D. Hogan, H. L. Falk, R. R. Bates, and I. Mitchel, *Science*, 1970, **168**, 864.

2 Primary Sources

Unlike many other environmental pollutants, PCDDs and PCDFs have never been manufactured deliberately, other than on the laboratory scale. A variety of primary sources are now known which contribute to environmental contamination and thus ultimately to contamination of foods. As noted above, the early concern was related to the formation of traces of 2,3,7,8-TCDD during manufacture of 2,4,5-trichlorophenol, leading to contamination in subsequent products such as 2,4,5-T and hexachlorophene. Still-bottom wastes from the manufacturing process were also often contaminated and appear to have been responsible for some of the pollution episodes in the USA, such as that at Times Beach. Controls were introduced in the UK in 1970 and 2,4,5-T has not been produced in Europe since 1983. Products containing this chemical are now of very limited availability and although environmental levels of 2,3,7,8-TCDD may still be partly attributable to past use of 2,4,5-trichlorophenol-based chemicals they are unlikely to be a significant current source.

Other chlorophenols, such as pentachlorophenol (PCP), may also be contaminated with PCDDs and PCDFs, although this principally involves the more highly chlorinated PCDD congeners; results from the analysis of some wood preservative formulations containing PCP have recently been published.[2] Polychlorinated biphenyls (PCBs) can become contaminated with PCDFs as a result of degradation during use and there have also been a number of fires involving PCB-filled equipment in which PCDFs have been formed. A broad range of chlorination levels and substitution patterns are normally involved. PCBs are no longer produced but much old electrical equipment containing PCBs continues in use and presents a risk of environmental pollution.

The presence of PCDDs and PCDFs in the fly ash of a municipal incinerator was reported in 1977,[3] and subsequent work has confirmed that emission of PCDDs and PCDFs is an almost inevitable consequence of such incineration.[4] Chemical waste incinerators, especially when used to destroy PCBs, are also potential sources as are hospital and other incinerators. Particular concern has recently been aroused by findings of high levels of PCDFs associated with wire reclamation plants.[5,6] Crummett[7] has suggested that any combustion process operating in the presence

[2] W. Christmann, K. D. Klöppel, H. Partscht, and W. Rotard, *Chemosphere*, 1989, **18**, 861.
[3] K. Olie, P. L. Vermeula, and O. Hutzinger, *Chemosphere*, 1977, **8**, 455.
[4] F. W. Karasek and O. Hutzinger, *Anal. Chem.*, 1986, **58**, 633A.
[5] A. Riss, H. Hagenmaier, U. Weberruss, C. Schlatter, and R. Wacker, 'Comparison of PCDD/PCDF Levels in Soil, Human Blood and Spruce Needles in an Area of PCDD/PCDF Contamination through Emissions from a Metal Reclamation Plant', Presented at 8th International Conference on Dioxins and Related Compounds, Umeå, 1988.
[6] A. Riss and H. Hagenmaier, 'Environmental Monitoring of PCDD/PCDF in the Vicinity of a Metal Reclamation Plant in the Tyrol/Austria', Presented at 9th International Conference on Dioxins and Related Compounds, Toronto, 1989.
[7] W. B. Crummett in 'Chlorinated Dioxins and Related Compounds – Impact on the Environment', ed. O. Hutzinger, R. W. Frei, E. Merian, and F. Focchiari, Pergamon Press, Oxford, 1982, p. 253.

of low concentrations of either inorganic or organic chlorides can be expected to produce traces of PCDDs and PCDFs – the 'trace chemistry of fire' hypothesis. There is increasing evidence for the *de novo* synthesis of these compounds in the absence of obvious precursors. Other combustion-related sources of PCDDs and PCDFs include exhaust emissions from vehicles fuelled by leaded petrol which normally contains chlorinated additives such as dichloroethane.[8–10] In contrast to chemical manufacturing sources, combustion processes produce almost all of the possible PCDD and PCDF congeners; the complexity of the resulting mixtures is illustrated in Figure 1. It should be noted that 2,3,7,8-TCDD typically comprises around 5% of the total TCDD produced.

A more recently discovered source which has attracted considerable attention is the chlorine bleaching of wood-pulp for paper making.[11–13] This leads to a characteristic pattern of TCDF isomers dominated by the 2,3,7,8- and 1,2,7,8-isomers with lesser amounts of many others. In addition 2,3,7,8-TCDD is normally present. This source has been of major concern in the Scandinavian countries and North America where pulp and paper making are practised on a large scale. Although there has been some attention to the contamination of foods, such as milk sold in cartons, by leaching from bleached pulp based packaging (see Section 5.4), and even over human exposure through direct contact with paper and other materials, it is the role of this source in contaminating the aquatic environment that is the main issue.

Some metallurgical processes have also been shown to act as sources, especially of PCDFs.[14] A plant in Norway where pellets of MgO mixed with coke are heated to about 700–800 °C in a chlorine atmosphere is estimated to produce an annual emission of 500 g 2,3,7,8-TCDD equivalents[15] (see Section 3.1 for a discussion of toxic equivalents calculations). In the past a nickel refining process involving high-temperature treatment of $NiCl_2$ is also thought to have produced significant amounts of TCDFs, although the current low-temperature process leads to relatively low emission rates.[15]

[8] K. Ballschmitter, H. Buchert, R. Niemczyk, A. Munder, and M. Swerev, *Chemosphere*, 1986, **15**, 901.
[9] S. Marklund, C. Rappe, M. Tysklind, and K. Egeback, *Chemosphere*, 1987, **16**, 29.
[10] S. Marklund, R. Andersson, M. Tysklind, C. Rappe, K.-E. Egebäck, E. Björkman, and V. Grigoriadis, *Chemosphere*, 1990, **20**, 553.
[11] US Environmental Protection Agency, 'The National Dioxin Study, Tiers 3, 5, 6 and 7', EPA 440/4-87-003, Office of Water Regulations and Standards, Washington, DC, 1987.
[12] S. E. Swanson, C. Rappe, J.Malmstrom, and K. P. Kringstad, *Chemosphere*, 1988, **17**, 681.
[13] G. Amendola, D. Barna, R. Blosser, L. LaFleur, A. McBride, F. Thomas, T. Tiernan, and R. Whittemore, *Chemosphere*, 1989, **18**, 1181.
[14] S. Marklund, L.-O. Kjeller, M. Hansson, M. Tysklind, C. Rappe, C. Ryan, H. Collazo, and R. Dougherty, in 'Chlorinated Dioxins and Dibenzofurans in Perspective', ed. C. Rappe, G. Choudhary, and L. Keith, Lewis Publishers, Michigan, 1986, p. 79.
[15] M. Oehme, S. Mano, and B. Bjerke, *Chemosphere*, 1989, **18**, 1379.

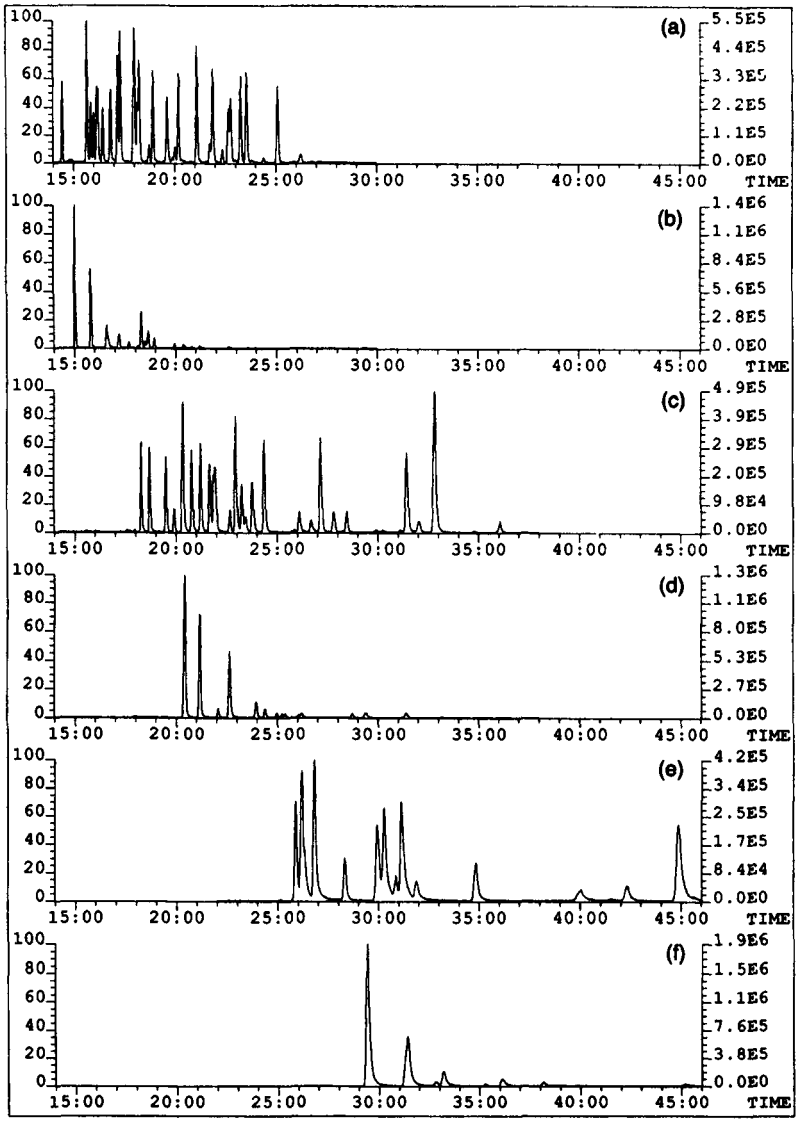


Figure 1 *Chromatograms for PCDDs and PCDFs having from 4 to 6 chlorine substituents in an extract of incinerator fly-ash. Analysis using a CPSil88 open-tubular GC column (Chrompack Ltd.) and mass spectrometric detection (selected ion monitoring):* (a) *TCDF (m/z 306);* (b) *TCDD (m/z 322);* (c) *PeCDF (m/z 338);* (d) *PeCDD (m/z 354);* (e) *HxCDF (m/z 374);* (f) *HxCDD (m/z 390)*

It has been reported that PCDDs and PCDFs can be formed from chlorophenols by *in vitro* enzymic oxidation with bovine lactoperoxidase or horseradish peroxidase.[16]

3 Toxicity and Health Effects

A number of reviews of the toxicity of PCDDs and PCDFs are available.[17-19] An early finding relating to 2,3,7,8-TCDD was the extraordinarily high acute oral toxicity of this compound in guinea-pigs, with an LD_{50} value of 0.6 $\mu g\,kg^{-1}$. A wide range of other toxic effects is caused in animals by 2,3,7,8-TCDD and other PCDDs and PCDFs. The effects noted include skin effects such as chloracne, organ damage (liver and thymus), immunological changes, haematological changes, reproductive toxicity, and carcinogenicity. The detailed assessment of carcinogenic risk remains controversial. According to the US EPA, 2,3,7,8-TCDD is the most potent carcinogen they have tested with an estimated dose associated with a 1 in 10^6 lifetime excess cancer risk of 0.006 pg/kg bodyweight/day,[20] but a recent alternative interpretation of the same data assesses the dose associated with this risk as 0.1 pg/kg bodyweight/day.[21] Other experts conclude that 2,3,7,8-TCDD is a non-mutagenic carcinogen for which it is appropriate to use a safety factor approach in extrapolating from animal carcinogenicity data to a possible level of concern for humans.[22]

Although acute lethality is clearly not a pertinent effect in considering the significance of trace environmental concentrations, LD_{50} values do provide a useful illustration of the very large inter-species differences in susceptibility which exist. In contrast to the value given above, that for the Golden Syrian hamster is 5051 $\mu g\,kg^{-1}$ and although the value for humans is unknown it is estimated to be very large. There are also very large differences in the potencies of different congeners. For example, the LD_{50} for 1,3,6,8-TCDD in the guinea-pig is in the g kg^{-1} range; this is one of

[16] L. G. Öberg, B. Glas, C. Rappe, and K. G. Paul, 'Biogenic Dioxin Formation', Presented at 9th International Conference on Dioxins and Related Compounds, Toronto, 1989.

[17] S. A. Skene, I. C. Dewhurst, and M. Greenberg, *Human Toxicol.*, 1989, **8**, 173.

[18] 'Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products', 2nd Edn., ed. R. D. Kimbrough and A. A. Jensen, Elsevier Science Publishers, Amsterdam and New York, 1989.

[19] 'Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans', Environmental Health Criteria No. 88, World Health Organization, Geneva, 1989.

[20] US Environmental Protection Agency, 'Health Assessment Document for Polychlorinated Dibenzo-*p*-dioxins and Polychlorinated Dibenzofurans', EPA/600/8-84/014F, Office of Health and Environmental Assessment, Washington, DC, 1985.

[21] R. E. Keenan, R. J. Wenning, A. H. Parsons, and D. J. Paustenbach, 'A Re-evaluation of the Tumor Histopathology of Kociba *et al.* (1978) Using 1990 Criteria: Implications for the Risk Assessment of 2,3,7,8-TCDD Using the Linearized Computer Multistage Model', Presented at 10th International Conference on Organohalogen Compounds, Bayreuth, 1990.

[22] Statement by the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment on the Human Health Hazards of Polychlorinated Dibenzo-*p*-dioxins and Polychlorinated Dibenzofurans. Included in 'Dioxins in the Environment – Report of an Interdepartmental Working Group' (Pollution Paper No. 27), HMSO, London, 1989.

the dominant components in the TCDD isomer pattern from combustion sources.

Although some of the different congeners may give rise to different biological effects and act through different mechanisms, it is now accepted that consistent effects and mechanisms of action are associated with the PCDD and PCDF congeners in which each of the 2-, 3-, 7-, and 8-positions are substituted – sometimes referred to as the laterally substituted congeners. There are in all 17 compounds (see Table 1) which satisfy this structural criterion and which give rise to similar effects to 2,3,7,8-TCDD, although with considerably different potencies. An important related fact is that these laterally substituted congeners are preferentially absorbed or retained in the fat stores of animals and, regardless of the mix of congeners to which exposure occurs, are the only ones normally found in human and other animal tissues, as illustrated by Figure 2.

Epidemiological studies of exposed human populations are still underway, but the evidence so far available regarding human health effects is regarded as reassuring and humans seem to be among the least sensitive species.[22] Certainly some of the people exposed in the Seveso accident have very high residual tissue levels without apparent ill-effect. It seems most unlikely that the often used description of 'dioxin' as 'the most toxic chemical known' is appropriate when considering human health effects.

3.1 Toxic Equivalence Schemes

Because PCDDs and PCDFs normally exist in environmental and biological samples as complex mixtures of congeners having different toxicities, the concept of 2,3,7,8-TCDD equivalents has been introduced to simplify risk assessment and regulatory control. The actual concentrations of the different congeners are multiplied by a 'toxic equivalency factor' (TEF) and summed to produce a 'TCDD equivalent' (TEQ) concentration. The approach assumes that synergistic and antagonistic effects do not exist. Although a number of different schemes of TEF values have been used over the past decade the most widely accepted values are those of the International Toxicity Equivalency Factor (I-TEF) model proposed by the Committee on the Challenges of Modern Society.[23] These factors are shown in Table 2. Values are ascribed for all of the 17 laterally substituted congeners; for other congeners the I-TEF value is 0. The I-TEF model has been accepted for use in the UK.[22]

The Nordic scheme[24] is also widely used and differs only in the factor of 0.01 ascribed to 1,2,3,7,8-pentaCDF. In biological samples this congener

[23] 'International Toxicity Equivalency Factor Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds, Pilot Study on International Information Exchange on Dioxins and Related Compounds', North Atlantic Treaty Organization/Committee on the Challenges of Modern Society, Report Number 176, 1988.

[24] 'Nordisk Dioxinriskbedömning, Rapport från en nordisk expertgrupp', Nord 1988:49, Nordisk Ministerråd, Copenhagen, 1988, p. 18.

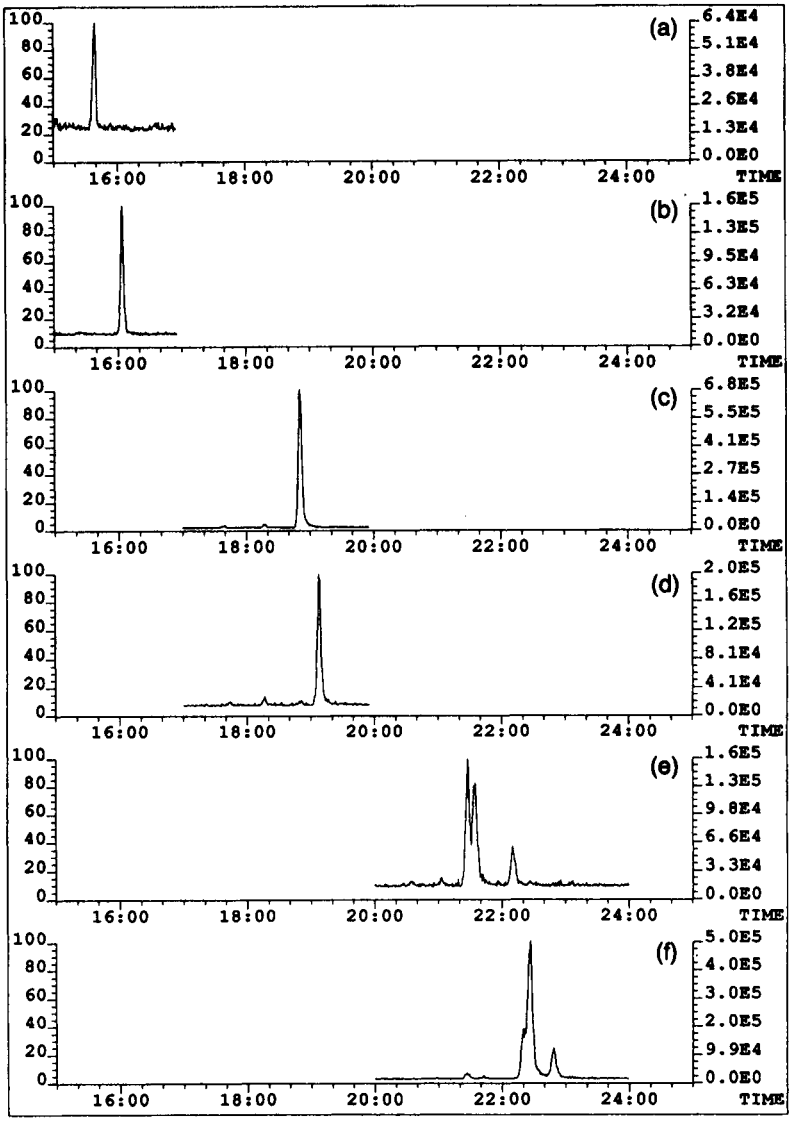


Figure 2 *Chromatograms for PCDDs and PCDFs having from 4 to 6 chlorine substituents in an extract of human milk. Analysis using a DB5 open-tubular GC column (Jones Chromatography Ltd.) and mass spectrometric detection (selected ion monitoring):* (a) *TCDF (m/z 306);* (b) *TCDD (m/z 322);* (c) *PeCDF (m/z 338);* (d) *PeCDD (m/z 354);* (e) *HxCDF (m/z 374);* (f) *HxCDD (m/z 390)*

Table 2 *The International Toxicity Equivalence Factors*

Congener	*I-TEF*
2,3,7,8-TCDD	1
1,2,3,7,8-PeCDD	0.5
1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,4,6,7,8-HpCD	0.01
OCDD	0.001
2,3,7,8-TCDF	0.1
2,3,4,7,8-PeCDF	0.5
1,2,3,7,8-PeCDF	0.05
1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
2,3,4,6,7,8,-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
OCDF	0.001

usually makes a very small contribution so that TEQ values calculated with the Nordic and I-TEF schemes differ very little. The scheme adopted by the German Federal Health Office assigns factors of 0.1 to 1,2,3,7,8-pentaCDD and to both 1,2,3,7,8-pentaCDF and 2,3,4,7,8-pentaCDF.[25] The difference of a factor of 5 in the weighting for the first and last of these congeners can make a substantial difference to the outcome of TEQ calculations for biological samples. Other schemes of historical and current interest are summarized in reference 23.

3.2 Guidelines for Maximum Intake

Various attempts have been made to assess acceptable levels of human exposure, or at least to assess the risk to health. In the UK the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment has recommended[22] that a figure of 1 pg/kg bodyweight/day be considered as a guideline value in the sense that this is a level which, when exceeded, should trigger investigation and appropriate measures to reduce environmental levels generally. In expressing this opinion the committee stated that, because of the considerable uncertainties inherent in deriving quantitative assessments, this should not be regarded as a tolerable daily intake.

[25] Umweltbundesamt (UBA), Sachstand Dioxine – Stand November 1984, Erich Schmidt Verlag, Berlin.

Limits which have been proposed or introduced in other countries include: Nordic Countries, 35 pg TCDD equivalents/kg bodyweight/week;[24] Netherlands, 4 pg/kg bodyweight/day; Federal Republic of Germany 1–10 pg/kg bodyweight/day;[25] Ontario, Canada 10 pg/kg bodyweight/day.[26] As pointed out by Barnes,[27] these doses were established by considering essentially the same evidence but applying different safety factors to the experimental 'no observable adverse effect levels'.

More recently a group of experts convened by the World Health Organization/Regional Office for Europe has recommended that the tolerable daily intake be set at 10 pg/kg bodyweight/day.[28]

There are very few examples of specific regulations or advice pertaining to particular dietary commodities. In Canada a 'Virtually Safe Dose' for 2,3,7,8-TCDD in fish of 20 ng kg^{-1} was established as a tolerance value,[29] and in the Netherlands an action level for milk of 6 ng kg^{-1} TEQ has been declared.[30]

4 Environmental Pathways

The entry point for PCDDs and PCDFs to the environment, as well as the pattern of congeners concerned, varies with the different sources. In the case of combustion the most direct route is through the atmosphere, where aerial transport of vapour-phase and particulate-bound material (such as fly ash) may result in dispersion of PCDDs and PCDFs over a considerable area before they settle or are washed by rain to ground level. PCDDs and PCDFs are of low volatility so that gas-phase transport is likely to be less important than adsorption on particulates. In the case of incinerators, PCDDs and PCDFs will also be present in the solid debris, such as grate ash, and can enter the environment when this is disposed of by landfill or other means.

Most of the PCDDs and PCDFs formed during pulp bleaching appear in the liquid effluent from the process and thus enter the aqueous environment directly. Again most of the PCDDs and PCDFs are likely to be particulate bound rather than in solution.

[26] Ontario Ministry of the Environment, Polychlorinated Dibenzo-*p*-dioxins and Polychlorinated Dibenzofurans, Scientific Criteria Document for Standard Development, No. 4-84, Toronto, Canada, 1985.

[27] D. G. Barnes, *Chemosphere*, 1989, **18**, 33.

[28] Consultation on tolerable daily intake from food of PCDDs and PCDFs, Report of meeting of WHO expert group, Bilthoven (4–7 December 1990), EUR/ICP/PCS 030(S), 21 December 1990.

[29] H. Tosine, 'Dioxins: a Canadian Perspective', in 'Chlorinated Dioxins and Dibenzofurans in the Total Environment', ed. G. Choudhary, L. H. Keith, and C. Rappe, Butterworth, Boston, 1983, p. 3.

[30] A. K. D. Liem, R. Hoogerbrugge, P. R. Kootstra, A. P. J. M. de Jong, J. A. Marsman, A. C. den Boer, R. S. den Hartog, G. S. Groenemeijer, and H. A. van't Klooster, 'Levels and Patterns of Dioxins in Cow's Milk in the Vicinity of Municipal Waste Incinerators and Metal Reclamation Plants in the Netherlands', Presented at 10th International Conference on Organohalogen Compounds, Bayreuth, 1990.

A further distribution mechanism that may be of considerable significance results from the presence of PCDDs and PCDFs in sewage sludge.[31-33] The primary sources have not been elucidated, but presumably could include contributions from industrial effluents, from that portion of the human dietary intake which is not absorbed, and from lavatory paper made from chlorine bleached pulp. It has not been established if PCDDs and PCDFs are actually formed in sewage, but the widespread use of chlorine-based bleaches must satisfy one of the prerequisite conditions. The evidence that biogenic formation is possible may also be of relevance.[16] Sewage sludge is disposed of by landfill and controlled sea dumping but is also used on agricultural land as a soil conditioner and fertilizer. One study has demonstrated elevated levels of PCDDs and PCDFs in soil as a result of regular sewage sludge application.[34] In Germany the application of sewage sludge to agricultural land has been restricted as a result of such findings.[34]

Clearly PCDDs and PCDFs will be present in surface waters as a result of direct contamination and atmospheric deposition; in sediments; in soils as a result of atmospheric deposition and the application of chemicals and sewage sludge; on surfaces as a result of atmospheric deposition; and, of course, in the air.

The concentrations found in urban air in a number of industrialized countries have been summarized by Travis,[35] and cover a remarkably narrow range from 1.3–9.6 $pg\,m^{-3}$. In the UK information is available on the levels of PCDDs and PCDFs in soil samples collected from points on a 50 km grid, thus defining the background levels.[36,37] This makes it clear that PCDDs and PCDFs are ubiquitous in the environment and occur in significantly higher concentrations in urban areas as compared with rural locations. Background levels for the TCDD homologue had a range of <0.5–69 $ng\,kg^{-1}$ with a median of 6.0 $ng\,kg^{-1}$ and levels of 2,3,7,8-TCDD were found to be in the range <0.5–2.1 $ng\,kg^{-1}$. In the case of urban soils the sum of the means for each homologue group was 2155 $ng\,kg^{-1}$ similar to the mean concentration of 1405 $ng\,kg^{-1}$ found in urban soils in the USA.[38]

[31] L. L. Lamparski, T. J. Nestrick, and V. A. Stenger, *Chemosphere*, 1984, **13**, 361.
[32] H. Hagenmaier, H. Brunner, W. Knapp and U. Weberruss, 'Untersuchungen der Gehalte an Polychlorierten Dibenzodioxinen, Polychlorierten Dibenzofuranen und Ausgewahlten Chlorkohlenwasserstoffen in Klärschlämmen', Forschungsbereicht 103 03 305, Im Auftrag des Umweltbundesamtes, 1988.
[33] C. Rappe, L.-O. Kjeller, and R. Anderson, *Chemosphere*, 1989, **19**, 13.
[34] M. S. McLachlan and M. Reissinger, 'The Influence of Sewage Sludge Fertilization on the PCDD/F Concentration in Soil', Presented at 10th International Conference on Organohalogen Compounds, Bayreuth, 1990.
[35] C. C. Travis and H. A. Hattemer-Frey, *Risk Analysis*, 1989, **9**, 91.
[36] C. S. Creaser, A. R. Fernandes, A. Al-Haddad, S. J. Harrad, R. B. Homer, P. W. Skett, and E. A. Cox, *Chemosphere*, 1989, **18**, 767.
[37] H. M. Inspectorate of Pollution, 'Determination of Polychlorinated Biphenyls, Polychlorinated Dibenzo-*p*-dioxins and Polychlorinated Dibenzofurans in UK Soils', Technical Report, HMSO, London, 1989.
[38] T. J. Nestrick *et al.*, *Chemosphere*, 1986, **15**, 1453.

A number of authors have used mathematical models to predict how PCDDs and PCDFs are partitioned in the environment and to suggest the major pathways of human exposure.[39–42] These studies tend to emphasize 2,3,7,8-TCDD, for which the most comprehensive physicochemical data are available. The calculations indicate that the predominant route of human exposure is through food – Travis,[39] for example, estimates 98% – and support the intuitive judgement that fatty foods are likely to be of greatest significance. In addition to these calculations the results of laboratory analysis of foods are becoming available.

5 Accumulation and Levels in the Food Chain

5.1 Vegetation

Plants are exposed to PCDDs and PCDFs via soil, groundwater, and by direct aerial deposition. Studies on uptake from soil, which have recently been reviewed,[43] are not entirely consistent. There is some evidence that the root systems of plants can absorb TCDD from soil but that this is concentrated in the outer surfaces of the roots;[44] passive uptake, in which soil particles are incorporated into the epidermis of the root tissue, has been suggested as a probable mechanism.[45] Most of the evidence suggests that there is no translocation within plants. PCDD and PCDF levels on foliage and fruits thus represent contributions from direct deposition of air-borne particulates and by absorption of vapour phase contaminants from the air including those which are attributable to evaporation from the soil.[46]

Not surprisingly, contamination of vegetation is associated with areas where there are specific PCDD and PCDF sources. High TCDD levels ($100\ ng\ kg^{-1}$) were detected in the peel of fruits grown on soils contaminated in the Seveso incident, but not in the flesh.[47] Recent results from the vicinity of a wire reclamation incinerator show considerable contamination ($5–10\ ng\ kg^{-1}$ TEQ) of leaf vegetables and rather less of fruits.[48] Apart

[39] C. C. Travis and H. A. Hattemer-Frey, *Chemosphere*, 1987, **16**, 2331.
[40] K. C. Jones and B. G. Bennett, *Sci. Total Environ.*, 1989, **78**, 99.
[41] T. E. McKone and P. B. Ryan, *Environ. Sci. Technol.*, 1989, **23**, 1154.
[42] J. B. Stevens and E. N. Gerbec, *Risk Analysis*, 1988, **8**, 329.
[43] G. A. Kew, J. L. Schaum, P. White, and T. T. Evans, *Chemosphere*, 1989, **18**, 1313.
[44] S. Facchetti, A. Balasso, C. Fichtner, G. Frare, A. Leoni, C. Mauri, and M. Vasconi, in 'Chlorinated Dioxins and Dibenzofurans in Perspective', ed. C. Rappe, G. Choudhary, and L. H. Keith, Lewis Publishers Inc., Michigan, 1986, p. 225.
[45] A. L. Young, 'Long Term Studies on the Persistence and Movement of TCDD in a Natural Ecosystem', in 'Human and Environmental Risks in Chlorinated Dioxins and Related Compounds', ed. R. E. Tucker, A. L. Young, and A. P. Gray, Plenum Press, New York, 1983, p. 173.
[46] A. Reischl, M. Reissinger, H. Thoma, and O. Hutzinger, *Chemosphere*, 1989, **19**, 467.
[47] H.-K. Wipf, E. Homberger, N. Neuner, U. B. Ranalder, W. Vetter, and J. P. Vuilleumier, in 'Chlorinated Dioxins and Related Compounds – Impact on the Environment', ed. O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari, Pergamon Press, Oxford, 1982, p. 115.
[48] B. Prinz, G. H. M. Krause, and L. Radermacher, 'Criteria for the Evaluation of Dioxins in Vegetable Plants and Soils', Presented at 10th International Conference on Organohalogen Compounds, Bayreuth, 1990.

from this localized contamination, levels of PCDDs and PCDFs in fruit and vegetables do not seem to make a significant contribution to intake although the amount of data relating to samples obtained at the retail level is limited. Although Davies[49] has published results for samples from Ontario, Canada, showing high levels in a fruit composite sample which consisted mainly of apples, more recent work with much more rigorous analytical quality control has shown levels in a variety of fruit and vegetable samples from this region to be below the limit of detection ($<1\ ng\,kg^{-1}$).[50] In a survey of foodstuffs available in West Berlin five vegetable samples were analysed (including cauliflower, lettuce, cherries, and apples) and again PCDDs and PCDFs were not found, subject to a detection limit of $0.01\ ng\,kg^{-1}$ for each isomer.[51] These were presumably background samples but it is not clear whether they were washed or whether outer leaves were discarded prior to analysis.

The levels of PCDDs and PCDFs in grass are also of interest since grazing animals, such as cattle and sheep, provide a link to the human diet through meat and dairy products. Preliminary results have been published[52] from a survey of levels in herbage in the UK using samples obtained from the same locations as the soil samples discussed above. The results so far available are for homologue group totals rather than specific congeners so that the toxic equivalents approach is not appropriate. In 67 samples the results for the TCDD group, for example, span the range $0.3–74.0\ ng\,kg^{-1}$ dry weight with an interquartile range of $2.3–9.7\ ng\,kg^{-1}$ and a median of $4.5\ ng\,kg^{-1}$; the contribution of 2,3,7,8-TCDD is estimated to be below $0.1\ ng\,kg^{-1}$. These results are clearly very similar to those cited for soils.

5.2 Animals

Grazing animals are potentially exposed to PCDDs and PCDFs through the ingestion of grass (or hay or silage), other feeds and water, and by inhalation. Intake calculations based on environmental modelling vary somewhat. Travis[39] suggests a typical daily intake by cattle of about $0.75\ ng\,day^{-1}$ 2,3,7,8-TCDD, while Stevens[42] has calculated the intake of all PCDDs to be in the region of $0.15\ ng\,day^{-1}$ for cattle in the vicinity of a municipal solid waste incinerator. Despite these differences in the quantitative assessment of dose both approaches lead to the conclusion that forage is the main source to the cattle. Travis estimates the contribution of forage and soil ingestion to be 80% and 20% respectively.

Laboratory studies have shown that 2,3,7,8-TCDD can be absorbed quite readily by the digestive system of animals; for rats a figure of 50–60% has been demonstrated,[53] and a study on a human volunteer

[49] K. Davies, *Chemosphere*, 1988, **17**, 263.
[50] B. Birmingham, B. Thorpe, R. Frank, R. Clement, H. Tosine, G. Fleming, J. Ashman, J. Wheeler, B. D. Ripley, and J. J. Ryan, *Chemosphere*, 1989, **19**, 507.
[51] H. Beck, K. Eckart, W. Mathar, and R. Wittkowski, *Chemosphere*, 1989, **18**, 417.
[52] J. R. Startin, M. Rose, and C. Offen, *Chemosphere*, 1989, **19**, 531.
[53] G. F. Fries and G. S. Marrow, *J. Agric. Food Chem.*, 1975, **23**, 265.

showed 87% absorption.[54] Distribution within body tissues favours fatty tissues, but lipid adjusted concentrations are usually fairly uniform. Although the dietary intake will, in most cases, include a complex mixture of congeners only the laterally substituted congeners persist in animal tissue.

5.2.1 *Meat*

Results from the analysis of retail samples of meat have been presented by Beck[51] and by Furst,[55] although these studies are based on very limited sample numbers. After adjusting for the differences between the I-TEF and German toxic equivalents schemes both of these studies indicate levels in beef, mutton, and chicken fat in the region of 1–5 ng kg^{-1} TEQ. It should be noted that these results are given on a fat weight basis and, since the fat content of the samples analysed is not reported, it is not possible to correct to a whole sample basis. Rather lower levels are reported for pork.

In an earlier study Ryan found a considerable incidence of hexa-, hepta-, and octa-chlorinated congeners in pork and chicken fat from various locations in Canada, associated with the use of PCP treated wood shavings as litter.[56]

5.2.2 *Milk*

In studies of lactating dairy cows fed a single dose of tritiated 2,3,7,8-TCDD, about 60% was eliminated in the faeces and about 13% appeared in the milk within 14 days, regardless of whether the TCDD was ingested with soil or with grain.[57,58] In an alternative approach with normal feed and under approximately steady-state conditions, McLachlan has shown that about 20% of ingested 2,3,7,8-TCDD appears in milk.[59] The proportion transferred decreased considerably with increasing chlorination level.

A number of reports have dealt with levels of PCDDs and PCDFs in cow's milk from various European countries.[30,51,55,60,61] The levels given in Table 3 for a number of farms in the UK are quite typical. In contrast a considerably lower background (0.006 ng kg^{-1} TEQ) has been reported in

[54] H. Poiger and C. Schlatter, *Chemosphere*, 1986, **15**, 1489.
[55] P. Furst, C. Furst, and W. Groedel, *Chemosphere*, 1990, **20**, 787.
[56] J. J. Ryan, R. Lizotte, T. Sakuma, and B. Mori, *J. Agric. Food Chem.*, 1985, **33**, 1023.
[57] D. Jones, S. Safe, E. Morcom, M. Holcomb, C. Coppock, and W. Ivie, *Chemosphere*, 1987, **16**, 1743.
[58] D. Jones, S. Safe, E. Morcom, M. Holcomb, C. Coppock, and W. Ivie, *Chemosphere*, 1989, **18**, 1257.
[59] M. S. McLachlan, H. Thoma, M. Reissinger, and O. Hutzinger, *Chemosphere*, 1990, **20**, 1013.
[60] C. Rappe, M. Nygren, G. Lindstrom, H. R. Buser, O. Blaser, and C. Wuthrich, *Environ. Sci. Technol.*, 1987, **21**, 964.
[61] J. R. Startin, M. Rose, C. Wright, I. Parker, and J. Gilbert, *Chemosphere*, 1990, **20**, 793.

Table 3 *Levels of PCDDs and PCDFs found in milk from rural locations in the UK (from ref. 61)*

Congener	ng kg^{-1} *whole milk*		
	Min	*Max*	*Mean*
2,3,7,8-TCDD	<0.004	0.013	0.009
1,2,3,7,8-PeCDD	0.012	0.023	0.016
1,2,3,4,7,8-HxCDD/ 1,2,3,6,7,8-HxCDD	0.019	0.043	0.032
1,2,3,7,8,9-HxCDD	<0.007	0.018	0.010
1,2,3,4,6,7,8-HpCDD	<0.022	0.066	0.046
OCDD	0.215	0.323	0.230
2,3,7,8-TCDF	<0.006	0.011	0.008
1,2,3,7,8-PeCDF	<0.002	<0.017	0.005
2,3,4,7,8-PeCDF	0.028	0.038	0.032
1,2,3,4,7,8-HxCDF	0.013	0.026	0.017
1,2,3,6,7,8-HxCDF	0.009	0.017	0.012
1,2,3,7,8,9-HxCDF	<0.002	<0.012	0.008
2,3,4,6,7,8-HxCDF	0.007	0.017	0.012
1,2,3,4,6,7,8-HpCDF	<0.007	<0.067	0.020
1,2,3,4,7,8,9-HpCDF	<0.010	<0.067	0.026
OCDF	0.023	0.071	0.041
Total TEQ	0.034	0.052	0.045

milk from New Zealand where the population is sparse and where municipal waste incineration is not practised.[62] A number of studies have shown a more localized influence of incinerators and other sources. In the Netherlands TEQ levels of 5–12 ng kg^{-1} fat have been found in the vicinity of one incinerator compared to 0.7–2.5 ng kg^{-1} from reference locations.[30] Samples from two farms in each of two locations in the UK where there are believed to be potential sources gave TEQ levels in the range 0.12–0.25 ng kg^{-1} whole milk.[61] The corresponding lipid adjusted range of 3.0–6.25 ng kg^{-1} is above background although the highest level is well below the highest found in the Netherlands.

The laterally substituted congeners are also predominant in milk, but some traces of other PCDDs and PCDFs are often evident, presumably because cows ingest the full range of combustion related congeners and because of the rapid distribution of intake to milk.

It should be noted that, as shown in Table 4, 2,3,4,7,8-PeCDF makes a major contribution to the total concentration measured as toxic equivalents.

[62] S. J. Buckland, D. J. Hannah, J. A. Taucher, and R. J. Weston, 'The Migration of Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans into Milks and Cream from Bleached Paperboard Packaging', Presented at 10th International Conference on Organohalogen Compounds, Bayreuth, 1990.

Table 4 *Contribution of individual PCDD and PCDF congeners to the total toxic equivalents (TEQ) concentration in milk, based on the mean levels recorded in Table 3 and the I-TEF factors given in Table 2*

Congener	*Contribution to TEQ total* (%)
2,3,7,8-TCDD	20
1,2,3,7,8-PeCDD	18
1,2,3,4,7,8-/1,2,3,6,7,8-HxCDD	7
1,2,3,7,8,9-HxCDD	2
1,2,3,4,6,7,8-HpCDD	1
OCDD	1
2,3,7,8-TCDF	2
1,2,3,7,8-PeCDF	1
2,3,4,7,8-PeCDF	36
1,2,3,4,7,8-HxCDF	4
1,2,3,6,7,8-HxCDF	3
1,2,3,7,8,9-HxCDF	2
2,3,4,6,7,8-HxCDF	3
1,2,3,4,6,7,8-HpCDF	< 1
1,2,3,4,7,8,9-HpCDF	1
OCDF	< 1

5.2.3 Eggs

There is very limited information on the incidence of PCDDs and PCDFs in hen's eggs. Beck has reported results for a single sample which gave a total concentration, in I-TEQ units, of 1.61 ng kg^{-1} on a fat weight basis.[51] Results have been reported from studies in the USA which show considerably higher levels in eggs from free-range birds as compared with those from more intensive conditions, and a correlation between levels in the eggs and those in soil.[63]

5.3 Fish

The accumulation of lipophilic chemicals in fish and transfer on to fish predators, including man, is well known. Although it is almost impossible to detect PCDDs and PCDFs in water these compounds can be found in fish, even from the oceans. A number of the studies of PCDD and PCDF levels in fish have dealt primarily with areas, such as the Great Lakes, with

[63] R. D. Stephens, M. Harnly, D. G. Hayward, R. R. Chang, J. Flattery, M. X. Petreas, and L. Goldman, *Chemosphere*, 1990, **20**, 1091.

a particular pollution history.[64–66] An extensive survey of fish from inland waters in the USA was conducted as part of a general programme of environmental screening.[67]

Some results are also available indicating the levels in fish as consumed in Western Europe.[51,55,61,68,69] In the UK the results from eight retail samples, including plaice, mackerel, herring, cod, skate, and coley, gave a mean of $0.74\,g\,kg^{-1}$ and a range of $0.15–1.84\,ng\,kg^{-1}$ TEQ on a wet weight basis.[61] Most of the other results available are broadly similar to those from the UK with the exception of the data from Sweden,[69] which show considerably more elevated levels in fish from lakes and from the Baltic Sea and the Gulf of Bothnia, presumably as a result of the polluted status of these waters.

The selectivity of uptake found in animals also applies to fish but is rather less complete. It is thus usual for the laterally substituted congeners to be the major PCDD and PCDF contaminants present, but for smaller amounts of other congeners also to be found. With crabs and some other crustaceans there seems to be little selectivity and the relative contributions of the different congeners in tissue represent those in the exposure. This is of some significance in attempting to attribute contamination to specific sources on the basis of source-characteristic congener patterns.[70]

5.4 Leaching from Paper and Paperboard

The formation of PCDDs and PCDFs during the chlorine bleaching of wood pulp can not only cause environmental contamination, but can also lead to these compounds being present in the finished paper and board.[71–73] Studies involving coffee filters showed them to contain

[64] J. J. Ryan, Pul-Yan Lau, J. C. Piulon, D. Lewis, H. A. McLeod, and A. Gervals, *Environ. Sci. Technol.*, 1984, **18**, 719.
[65] N. V. Fehringer, S. M. Walthers, R. J. Kozara, and L. F. Schneider, *J. Agric. Food Chem.*, 1985, **33**, 626.
[66] D. De Vault, W. Dunn, P.-A. Bergqvist, K. Wiberg, and C. Rappe, *Environ. Toxicol. Chem.*, 1989, **8**, 1013.
[67] D. W. Kuehl, B. C. Butterworth, A. McBride, S. Kroner, and D. Bahnick, *Chemosphere*, 1989, **18**, 1997.
[68] A. Biseth, M. Oehme, and K. Faerden, 'Levels of Polychlorinated Dibenzo-*p*-dioxins and Polychlorinated Dibenzofurans in Selected Norwegian Foods', Presented at 10th International Conference on Organohalogen Compounds, Bayreuth, 1990.
[69] C. de Wit, B. Jansson, M. Strandell, M. Ohlsson, S. Bergek, M. Boström, P.-A. Bergqvist, C. Rappe, and Ö. Andersson, 'Polychlorinated Dibenzo-*p*-dioxin and Dibenzofuran Levels and Patterns in Fish Samples Analyzed within the Swedish Dioxin Survey', Presented at 10th International Conference on Organohalogen Compounds, Bayreuth, 1990.
[70] M. Oehme, A. Bartnova, and J. Knutzen, *Environ. Sci. Technol.*, 1990, **24**, 1836.
[71] H. Beck, K. Eckart, W. Mathar, and R. Wittkowski, *Chemosphere*, 1988, **17**, 51.
[72] V. H. Kitunen and M. S. Salkinoja-Salonen, *Chemosphere*, 1989, **19**, 721.
[73] K. Wiberg, K. Lundström, B. Glas, and C. Rappe, *Chemosphere*, 1989, **19**, 735.
[72] V. H. Kitunen and M. S. Salkinoja-Salonen, *Chemosphere*, 1989, **19**, 721.
[73] K. Wiberg, K. Lundström, B. Glas, and C. Rappe, *Chemosphere*, 1989, **19**, 735.

1–5 ng kg^{-1} 2,3,7,8-TCDD and 10–30 ng kg^{-1} 2,3,7,8-TCDF.[74,75] Although the evidence suggests that a considerable proportion, 30% or more, can be extracted during coffee brewing the resulting concentrations in coffee remain very low. A risk assessment of the potential exposure to PCDDs and PCDFs associated with the consumption of tea brewed with bleached-pulp tea bags has concluded that this is negligible.[76]

It has also been shown that some leaching can occur from paperboard milk cartons into milk, despite the presence of an interior polyethylene surface coating.[77–80,61] The evidence suggests that some 10–20% of the 2,3,7,8-TCDF in the carton material is transferred to milk on storage for up to 12 days. It is rather less clear whether or not equivalent migration of 2,3,7,8-TCDD also occurs. The results from Canada[77] and New Zealand[78] suggest that this migration contributes substantially to the total concentration in the milk. In Europe, however, where the background levels in milk are somewhat greater and levels in the cartons are typically lower, migration accounts for a smaller proportion of the typical final concentrations. The introduction of improved pulp production technology, which produces markedly lower PCDD and PCDF concentrations,[81] is likely to greatly reduce this route of human exposure.

6 Levels in Human Milk and Tissue

In general, results on human tissue are remarkable more for the uniformity of the background levels than for the differences. For 2,3,7,8-TCDD, for which the greatest quantity of data is available, adipose tissue levels for individuals from industrialized countries fall in the range 3–20 ng kg^{-1} with a mean of 7 ng kg^{-1}.[40]

One study, in Sweden, has demonstrated a clear difference between a group of fish-allergic individuals, a control group, and a group with a high

[74] H. Beck, A. Dross, K. Eckart, W. Mathar, and R. Wittkowski, *Chemosphere*, 1989, **19**, 655.

[75] National Council of the Paper Industry for Air and Stream Improvement Inc., 'Assessment of the Risks Associated with the Potential Exposure to Dioxins through the Consumption of Coffee Brewed Using Bleached Paper Coffee Filters', NCASI Technical Bulletin 546, New York, 1988.

[76] M. J. Sullivan and J. T. Stanford, *Chemosphere*, 1990, **20**, 1755.

[77] J. J. Ryan, L. G. Panopio, and D. A. Lewis, 'Bleaching of Pulp and Paper as a Source of PCDDs and PCDFs in Food', Presented at 8th International Symposium on Dioxins and Related Compounds, Umeå, 1988.

[78] S. J. Buckland, D. J. Hannah, J. A. Taucher, and R. J Weston, 'The Migration of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans into Milks and Cream from Bleached Paperboard Packaging', Presented at 10th International Conference on Organohalogen Compounds, Bayreuth, 1990.

[79] L. LaFleur, T. Bousquet, K. Ramage, B. Brunck, T. Davis, W. Luksemburg, and B. Peterson, *Chemosphere*, 1990, **20**, 1657.

[80] C. Rappe, G. Lindström, B. Glas, and K. Lundström, *Chemosphere*, 1990, **20**, 1649.

[81] V. H. Kitunen and M. S. Salkinoja-Salonen, *Chemosphere*, 1990, **20**, 1663.

fish consumption.[82] There is, however, no suggestion of any health effect being attributable to this difference in exposure.

Human milk has been of considerable interest, partly because it is the sole food source for many infants, but also because it is a non-invasive way of looking at levels in human tissue. A unique initiative by the World Health Organization/Regional Office for Europe has made available results from many regions of the world in a study featuring a comprehensive scheme of interlaboratory calibration.[83] The average levels in milk from women living in widely dispersed locations in Western Europe were remarkably similar and in the range 30–40 $ng\,kg^{-1}$ (TEQ total on a fat basis). Rather lower levels were reported in samples from some Nordic countries.

7 Analytical Considerations

As might be expected in view of the very low concentrations concerned and the complexity of the mixtures of congeners, the analysis of PCDDs and PCDFs in foods and other biological samples is challenging. Successful analysis of the minute concentrations present in biological samples and in foods inevitably requires access to a high-performance mass spectrometer and needs extensive and time-consuming sample clean-up. Many different variations of methodology are available and these have been summarized in a recent comprehensive review.[84] The use of stable isotope internal standards is now regarded as an essential step for correct measurement. Guidelines for the validation and quality control of analytical work have recently been proposed.[85]

There are a number of points which need to be clearly appreciated in assessing the reported data. The analytical standards, on which the accuracy of quantification depends, are normally available only as dilute solutions and there must be some concern as to the initial and long-term accuracy of these solutions. There are some unfortunate differences of practice in reporting results on a whole sample or lipid basis which can hamper comparison. The effects of the different toxic equivalent schemes must be considered. The level of some individual congeners are often close to the analytical detection limit and may be subject to considerable imprecision in measurement. In calculating total levels in toxic equivalent units there are differences of approach in accounting for congeners which have not been detected. In some reports the actual level is assumed to be

[82] B.-G. Svensson, A. Nilsson, M. Hansson, C. Rappe, B. Akesson, and S. Skerfving, 'Fish Consumption and Human Exposure to Dioxins and Dibenzofurans', Presented at 10th International Conference on Organohalogen Compounds, Bayreuth, 1990.

[83] 'Levels of PCBs, PCDDs and PCDFs in Breast Milk', *Environ. Health*, **34**, World Health Organization/Regional Office for Europe, Copenhagen, 1989.

[84] R. E. Clement and H. M. Tosine, *Mass Spectrom. Rev.*, 1988, **7**, 593.

[85] P. F. Ambidge, E. A. Cox, C. S. Creaser, M. Greenberg, M. G. de Gem, J. Gilbert, P. W. Jones, M. G. Kibblewhite, J. Levey, S. G. Lisseter, T. J. Meredith, L. Smith, P. Smith, J. R. Startin, I. Stenhouse, and M. Whitworth, *Chemosphere*, 1990, **21**, 999.

zero, and in others to be 50% or 100% of the detection limit. It must also be recognized that real detection limits vary between analyses and between individual congeners in a single analysis. Unless great care is taken in the interpretation of results it is possible for differences in analysis and reporting to cause apparent differences in the PCDD and PCDF levels found.

8 Conclusion

It is clear that, although food is the primary source of PCDDs and PCDFs to man, the extent to which foods contain these compounds is not a product of agricultural or food manufacturing practice but depends almost entirely on the general level of environmental contamination. Compared with the concentrations of many other man-made and natural toxins PCDDs and PCDFs are present at extremely low levels. There is no clear evidence that these small levels of exposure pose a significant risk to human health, although it is impossible to rule this out entirely. The presence of an entirely man-made contaminant in the environment and in food is undesirable but the only feasible approach to control is by the abatement or elimination of the primary sources. Nevertheless a programme of monitoring to better identify and quantify the sources and to gain further information on average and extreme intakes is likely to remain necessary.

CHAPTER 3

Analysis and Occurrence of Polycyclic Aromatic Hydrocarbons in Food

KEITH D. BARTLE

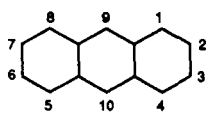
1 Nature and Biological Action of Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) comprise the largest class of known environmental carcinogens;[1] some, while not carcinogenic, may act as synergists. PAHs are found in water, air, soil, and, therefore, food; they originate from diverse sources such as tobacco smoke, engine exhausts, petroleum distillates, and coal-derived products, with combustion sources predominating.[2–4] PAHs in food may also arise from smoke curing, charcoal broiling, food additives, and packaging as well as environmental pollution.[5]

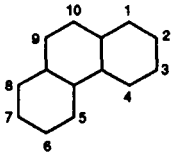
PAHs consist[6] (Figure 1) of benzene units linked together, either cata-annellated [*e.g.* anthracene (1), linearly annellated, and phenanthrene (2), angularly annellated] or peri-condensed [*e.g.* pyrene (3)]. These possibilities, taken with the number of alkylated derivatives, make the

[1] 'Environmental Carcinogens: Polycyclic Aromatic Hydrocabons', ed. G. Grimmer, CRC Press, Boca Raton, Florida, 1983.
[2] M. L. Lee. M. V. Novotny and K. D. Bartle, 'Analytical Chemistry of Polycyclic Aromatic Compounds', Academic Press, New York, 1981, Chapter 2, p. 17.
[3] A. Bjørseth and T. Ramdahl, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjørseth and T. Ramdahl, Marcel Dekker, New York, 1985, Vol. 2, Chapter 1, p. 1.
[4] T. Vo-Dinh, in 'Chemical Analysis of Polycyclic Aromatic Compounds', ed. T. Vo-Dinh, John Wiley, New York, 1989, Chapter 1, p. 1.
[5] T. Fazio and J. W. Howard, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjørseth, Marcel Dekker, New York, 1983, Vol. 1, Chapter 11, p. 461.
[6] M. Zander, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjørseth, Marcel Dekker, New York, 1983, Vol. 1, Chapter 1, p. 1.

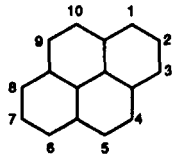
number of isomers for even a modest molecular weight enormous (Table 1) and PAH mixtures from combustion *etc.* are usually extremely complex.



(1)



(2)



(3)

Figure 1

Table 1 *Number of possible PAHs with n six-membered rings*[a]

n	*Cata-annellated*	*Peri-condensed*	*Total*
1	1	0	1
2	1	0	1
3	2	1	3
4	5	2	7
5	12	10	22
6	37	45	82
7	123	210	333

[a] From M. Zander, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjørseth, Vol. 1. Marcel Dekker, New York, 1983, p. 3.

Not all PAHs show biological activity, and there is often significant variation between isomers. Table 2 compares the carcinogenic activity[7] of selected PAHs. Modern testing tends towards the use of bacterial mutation (Ames test) rather than the induction of tumours in animals; virtually all carcinogenic compounds are mutagenic, although not all mutagenic compounds have been demonstrated to be carcinogenic. Minor constituents of PAH mixtures, such as alkyl derivatives, may make large contributions to the carcinogenic activity of the total mixture. For example, Table 3 shows how certain methylchrysenes, particularly the 5-isomer – one of the most carcinogenic compounds known[8] – may dominate the activity of a given fraction.[9,10]

[7] International Agency for Research on Cancer, 'IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Polynuclear Aromatic Hydrocarbons Part I', IARC, Lyon, France, 1983.

[8] S. S. Hecht, M. Loy, R. Mazzarese, and D. Hoffman, in 'Polycyclic Aromatic Hydrocarbons and Cancer', ed. H. V. Gelboin and P.O.P. Ts'o, Academic Press, New York, 1978, Vol. 1, p. 119.

[9] S. S. Hecht, W. E. Bondinell, and D. Hoffman, *J. Nat. Cancer Inst.*, 1974, **53**, 1121.

[10] N. A. Goeckner and W. M. Griest, *Sci. Total Environ.*, 1977, **8**, 187.

Table 2 *Evaluation[a] of the carcinogenic activity of selected PAHs*

Compound	*Evidence of carcinogenicity in experimental animals*	*Evidence from short term tests*	*Mutagenicity to Salmonella typhimurium (Ames Test)*
Fluoranthene	None	Limited	Positive
Pyrene	None	Limited	Positive
Benz[*a*]anthracene	Limited	Sufficient	Positive
Chrysene	Limited	Limited	Positive
Benzo[*b*]fluoranthene	Sufficient	Inadequate	Positive
Benzo[*a*]pyrene	Sufficient	Sufficient	Positive
Benzo[*ghi*]perylene	Inadequate	Inadequate	Positive
Dibenz[*a,h*]anthracene	Limited	Sufficient	Positive

[a] From A. Bjørseth and G. Becher, 'Polycyclic Aromatic Hydrocarbons in Workplace Atmospheres: Occurrence and Determination', CRC Press, Boca Raton, Florida, 1986, p. 3.

Table 3 *Comparison of the contents of chrysene and its methyl derivatives in environmental samples[a]*

	Relative Carcinogenicity	*Cigarette smoke, ng per cigarette*	*Coal liquid p.p.m.*
Chrysene	+	37	98
1-Methylchrysene	0/+	3	ND
2-Methylchrysene	++	1	102
3-Methylchrysene	++	6	106
4-Methylchrysene	++	0	19
5-Methylchrysene	+++	0.5	
6-Methylchrysene	++	7	64

[a] From M. L. Lee, M. V. Novotny, and K. D. Bartle, 'Analytical Chemistry of Polycyclic Aromatic Compounds', Academic Press, New York, 1981, p. 356.

In recent years significant progress has been made in the understanding of the biological action of PAHs.[4,11] These compounds enter the body by inhalation and ingestion and are distributed to various organs after adsorption by phospholipids and/or complexation by serum albuminates. Enzymatic oxidation by mixed function oxidases (MFO), often termed aryl hydrocarbon hydroxylases, which are most abundant in the liver, followed by hydrolysis to dihydrodiols by aryl epoxide hydrolysases, then occurs. The products are the true active species, the 'ultimate carcinogens', the so-called 'bay region' dihydrodiol epoxides. These compounds form covalent adducts with proteins and nucleic acids. The DNA adducts are thought to initiate cell mutation and eventual malignancy (Figure 2).

[11] A. Bjørseth and G. Becher 'Polycyclic Aromatic Hydrocarbons in Workplace Atmospheres: Occurrence and Determination', CRC Press, Boca Raton, Florida, 1986, Chapter 7, p. 103.

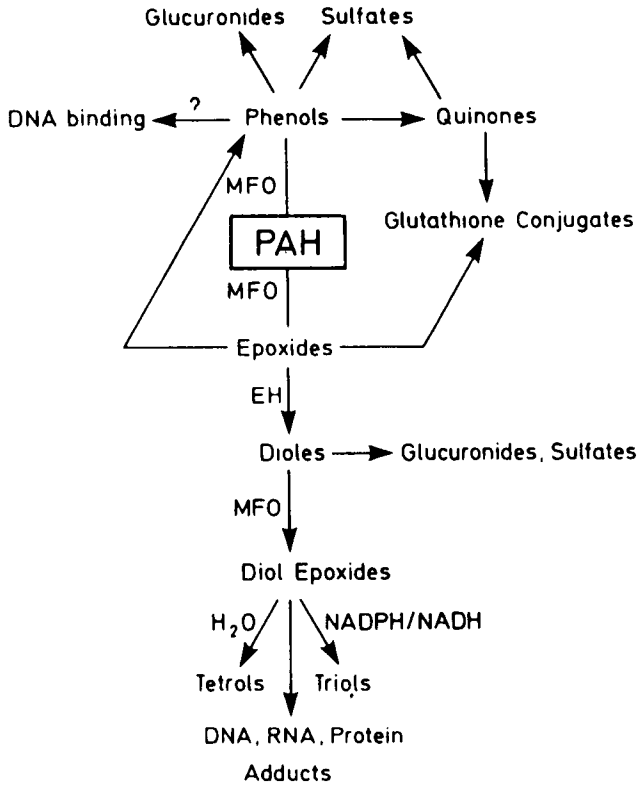


Figure 2 *Enzymatic pathways involved in the activation and detoxification of PAHs: MFO, multifunctional mono-oxygenase, EH, epoxide hydrolase*
(Reproduced with permission from A. Bjørseth and G. Becher 'Polycyclic Aromatic Hydrocarbons in Workplace Atmospheres: Occurrence and Determination', CRC Press, Boca Raton, Florida, 1986)

The formation of PAH-derivative–DNA adducts has been demonstrated by a variety of immunochemical[12] and spectroscopic techniques[4] and followed by HPLC making use of fluorescence.[13] Other reactions involved in PAH metabolism include conjugation[14] of oxygenates (*e.g.* phenols) to glucuronic sulphates and to give water-soluble compounds which are excreted; 1-hydroxypyrene has been shown[15] to be a major metabolite present in urine.

[12] R. M. Santella, L. L. Hsieh, C. D. Lin, S. Viet, and I. B. Weinstein *Environ. Health Perspect.*, 1985, **62**, 95.
[13] R. O. Rahn, S. S. Chang, J. M. Holland, and L. R. Shugart, *Biochem. Biophys. Res. Commun.*, 1982, **109**, 262.
[14] N. Nemoto, in 'Polycyclic Aromatic Hydrocarbons and Cancer', ed. H. V. Gelboin and P. O. P. Ts'o, Academic Press, New York, 1981, Vol. 3, p. 213.
[15] S. D. Keimig, K. W. Kirby, D. P. Morgan, J. F. Keiser, and T. D. Huberg, *Xenobiotica*, 1983, **13**, 415.

2 Origin of PAHs in Food

PAHs in food originate mainly in: the preparation, particularly smoking, and cooking process; by deposition on to vegetable matter of environmental pollutants; or concentration from water pollutant PAH. The relative importance of these sources is discussed in Section 4.

Formation of PAHs is favoured[16,17] by pyrolysis or air-deficient combustion of organic matter at temperatures in the range 500–900 °C and especially above 700 °C. Since peptides, lipids, carbohydrates, terpenes, and leaf pigments have all been shown[2] to be precursors, it is not surprising that cooking at high temperatures under conditions in which pyrolysis products remain in contact with the food leads to the presence of PAHs. The greatest concentrations of PAHs have been shown to arise from the pyrolysis of lipids, especially sterols.[17]

Since incomplete combustion of wood generates PAHs in large quantities,[18] smoked food products are also inevitably contaminated with PAHs. Less avoidable are the PAHs which originate in the environment. There is strong evidence that the origin of PAHs in many foods of vegetable origin lies in atmospheric deposition directly on to the above-ground plant,[19] and that uptake from the soil is of minor importance. Whenever fossil fuels, waste, or vegetation are burned PAHs are released and dispersed into the atmosphere, from which they are deposited directly on to plants.

In definitive work, Jones *et al.* analysed[20,21] grass and wheat grain stored from agricultural experiments conducted on the same site since the 1840s for 16 PAHs. The values for wheat grain harvested between 1979 and 1986 were closely similar to those reported for cereals[22] by Dennis *et al.* in 1983. The patterns of PAHs detected were similar over the extended time series studied, in spite of changing patterns of fuel use, apparently because of the influence of biodegradation, photo-oxidation, and vaporization during transport and retention of PAHs in the environment. A very marked decline in PAHs in grass samples since the 1920s, with corresponding, though lesser, declines for wheat grain parallels the improvement in UK air quality over the same period.

The origin of large amounts of PAHs in certain foods of marine origin is less thoroughly studied. It seems likely, however, that this is most likely to

[16] J. P. Longwell in 'Nineteenth Symposium (International) on Combustion', The Combustion Institute, 1982, p. 1339.

[17] I. Schmeltz and D. Hoffman, in 'Carcinogenesis – A Comprehensive Survey', ed. R. I. Freidenthal and P. W. Jones, Raven Press, New York, 1976, Vol. 1, p. 225.

[18] D. G. DeAngelis, D. S. Ruffin, J. A. Peters, and R. B. Reznik, 'Source Assessment: Residential Combustion of Wood', EPA-600/2-80-0426, Research Triangle Park, North Carolina, 1980.

[19] G. Grimmer and D. Duvel, *Z. Naturforsch.*, 1970, **256**, 1171.

[20] K. C. Jones, J. A. Stratford, K. S. Waterhouse, E. T. Furlong, W. Giger, R. A. Hites, C. Schaffner, and A. E. Johnston, *Environ. Sci. Technol.*, 1989, **23**, 95.

[21] K. C. Jones, G. Grimmer, J. Jacob, and A. E. Johnston, *Sci. Total Environ.*, 1989, **78**, 117.

[22] M. J. Dennis, R. C. Massey, D. J. McWeeny, M. E. Knowles, and D. Watson, *Food Chem. Toxicol.*, 1983, **21**, 569.

lie in the concentration by shellfish of traces of pollutant PAHs in sea water. Levels two or three orders of magnitude above background have been reported[23] in lobsters from areas receiving discharges from coal and coking plants.

3 Analysis of PAHs in Food

Individual PAHs are generally present in foods at the p.p.b. ($\mu g\,kg^{-1}$) level. Three steps are therefore most often involved in analysis: extraction and clean-up, chromatographic separation, and spectroscopic identification.

3.1 Extraction and Clean-up

Extraction of PAHs from oils and fats is straightforward if the materials are soluble in a solvent such as cyclohexane. Recoveries near 100% for PAHs added at the 2 and 10 p.p.b. level have been demonstrated.[24,25] PAHs may be obtained from fruit and vegetable matter by Soxhlet extraction or sonication with a suitable solvent.[26] For insoluble fats and protein-rich foods such as meat, fish, and cheese, alkaline saponification to release PAHs bound to food components is necessary;[27] the samples are then extracted into a hydrocarbon solvent. Thus, only about 30% of PAH is extractable from smoked herring without saponification, which increases the extraction yield to almost 100%.[25]

The food extracts so obtained inevitably contain substantial quantities of interfering materials other than PAHs. Enrichment and clean-up are usually carried out by solvent partition followed by column chromatography. Extraction into dimethylformamide[28] followed by precipitation by water addition and back extraction has been widely applied.[25] Further clean-up on silica gel and separation of PAH fractions on Sephadex LH-20 gel is recommended (Figure 3).[25,28]

3.2 Analysis of the PAH Fraction

The PAH fraction isolated from food is a complex mixture of numerous individual compounds: 146 different PAHs have been identified in automobile emissions,[29] and *ca.* 70 in air particulates.[30,31] The highest resolution

[23] C. J. Musial and J. F. Uthe, *J. Assoc. Off. Anal. Chem.*, 1986, **69**, 462.
[24] J. W. Howard, E. W. Turricchi, R. H. White, and T. Fazio, *J. Assoc. Off. Anal. Chem.*, 1966, **49**, 1237.
[25] G. Grimmer and H. Böhnke, *J. Assoc. Off. Anal. Chem.*, 1975, **58**, 725.
[26] J. T. Coates, A. W. Elzerman, and A. W. Garrison, *J. Assoc. Off. Anal. Chem.*, 1986, **69**, 110.
[27] J. W. Howard, R. T. Teague, R. H. White, and B. E. Fry, jr., *J. Assoc. Off. Anal. Chem.*, 1966, **49**, 595.
[28] M. Novotny, M. L. Lee, and K. D. Bartle, *J. Chromatogr. Sci.*, 1974, **12**, 606.
[29] G. Grimmer, H. Böhnke, and A. Glaser, *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt.*, 1977, **164**, 218.
[30] M. L. Lee, M. Novotny, and K. D. Bartle, *Anal. Chem.*, 1976, **48**, 1566.
[31] A. Bjørseth and G. Eklund, *Anal. Chim. Acta*, 1979, **105**, 199.

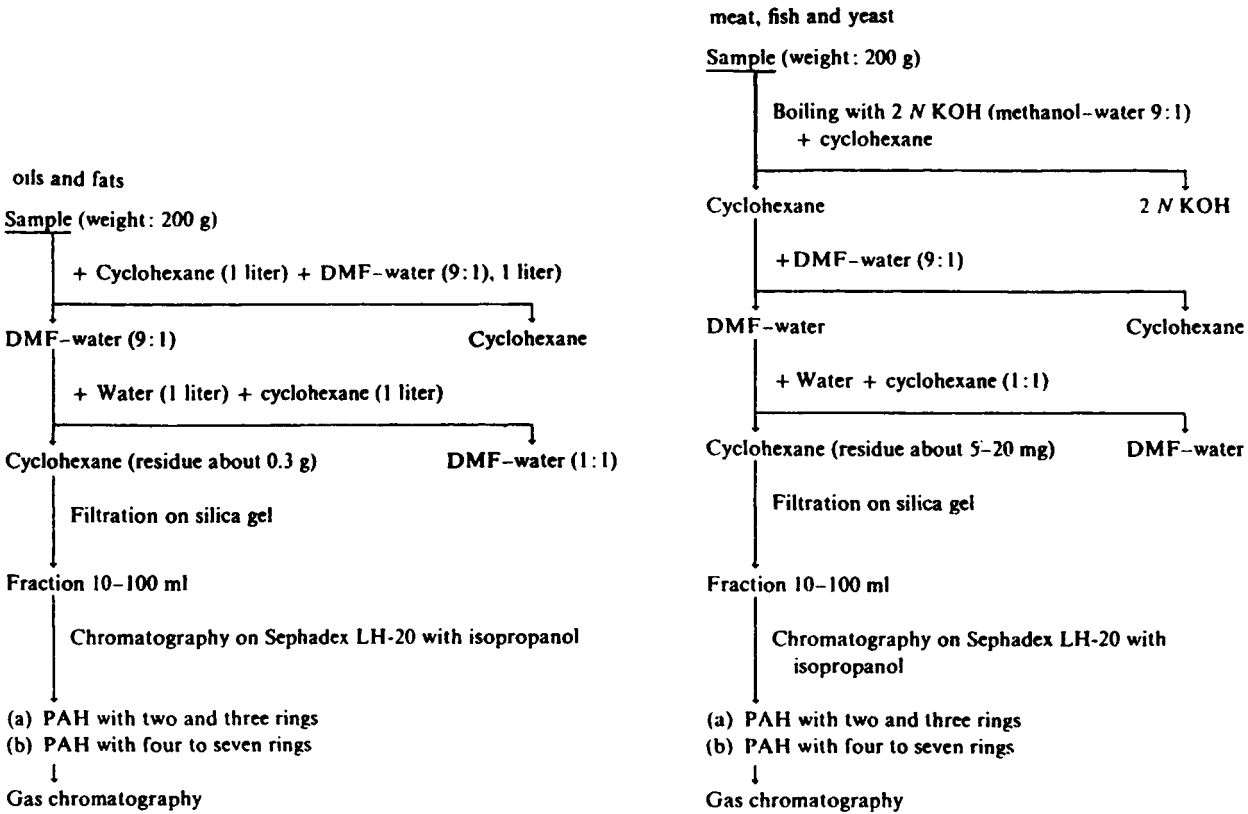


Figure 3 *Schemes for extraction and concentration of PAHs*
(Reproduced with permission from G. Grimmer and H. Böhnke, *J. Assoc. Offic. Anal. Chem.*, 1975, **58**, 725)

possible is therefore required,[28] followed by spectroscopic identification; the methods most generally applied in PAH analysis are thin-layer (TLC), high performance liquid (HPLC), capillary gas (CGC), and most recently, supercritical fluid chromatography (SFC).

3.2.1 TLC

TLC is rapid and simple to apply, and separated components may be recovered for further analysis; the main difficulty is oxidation of PAHs on the active stationary phase. Among available TLC systems,[32] cellulose with 1:1 dimethylformamide/water mobile phase gives the widest range of retention, and isomer pairs may be further separated by changing the water content. Two-dimensional dual band TLC has been shown[33] to give very high resolution of PAHs (*e.g.* Figure 4): the TLC plate consists of alumina and acetylated cellulose layers, and separation occurs first on the alumina (n-hexane/ether mobile phase), with further separation on the second layer with methanol–ether–water. Quantification of PAHs in TLC may be achieved by *in situ* scanning fluorescence densitometry;[32] alternatively separated spots may be isolated from the plate by sublimation or solvent extraction.

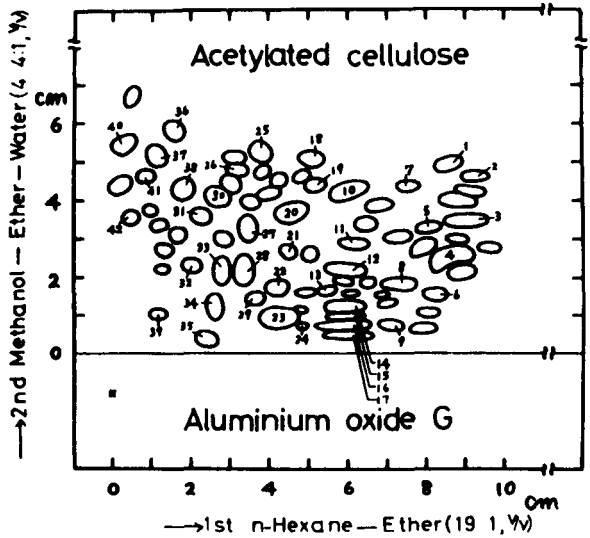


Figure 4 *Two-dimensional dual-band thin-layer chromatogram of pollutant PAH mixture*
(Reproduced with permission from H. Matsushita 'Polycyclic Aromatic Hydrocarbons and Cancer', Vol. 1, ed. H. V. Gelboin and P.O.P. Ts'o, Academic Press, New York, 1978)

[32] J. M. Daisey, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjørseth, Marcel Dekker, New York, 1983, Vol. 1, p. 397.
[33] H. Matsushita, in 'Polycyclic Aromatic Hydrocarbons and Cancer', ed. H. V. Gelboin and P. O. P. Ts'o, Academic Press, New York, 1978, Vol. 1, p. 71.

3.2.2 HPLC

Several advantages are afforded by HPLC in the analysis of PAHs: selectivity, sensitive and selective detection, and fractionation before the application of other chromatographic or spectroscopic techniques.[34–36]

Selectivity in HPLC is conferred by interactions of solute with both stationary and mobile phases, while UV absorption and fluorescence spectroscopy afford excellent detection methods for PAHs. However, HPLC does not approach the separation efficiency of capillary GC.

Reverse phase separation of PAHs on chemically bonded alkyl silica stationary phases (*e.g.* C_{18}) is the most popular HPLC mode and is preferred over normal phase HPLC. The mobile phase is usually a mixture of a polar solvent and water, the composition of which can be varied in gradient elution. Polymeric phases are most effective,[35] and give separations (Figure 5) in which the shape of the PAH molecule plays an important role.[35,36] Alkyl group number and position also influence retention in reverse phase HPLC. Figure 6 shows representative reverse phase HPLC chromatograms of the PAH fractions from a number of smoked foods.[37]

3.2.3 Capillary GC

The greatest chromatographic resolution available is achieved by capillary GC; the availability of robust fused silica columns, usually 10–25 m long and 0.2–0.3 mm internal diameter, with well deactivated surfaces and cross-linked thermostable stationary phases, now makes this the method of choice for PAH analysis.[36,38,39] Cross-linked polysiloxanes such as SE-54 (methylpolysiloxane with 5% phenyl groups) are most usually employed as stationary phases, but the recent availability of phases with liquid crystalline substituents has given rise to remarkable separations[36] of isomers on the basis of molecular shape – especially the length-to-breadth ratio.

Although flame ionization is the most commonly applied detector in GC of PAHs because of its excellent linearity, sensitivity, and reliability, a range of more sensitive and selective detectors may be used: the electron capture (ECD) and photoionization detectors both exhibit[39] marked selectivity for PAHs. Capillary GC with mass spectrometric detection (GC/MS) represents[40] a powerful combination for PAH analysis. Monitoring of the

[34] S. A. Wise, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjørseth, Marcel Dekker, New York, 1983, Vol. 1, Chapter 5, p. 183.
[35] S. A. Wise, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjørseth and T. Ramdahl, Marcel Dekker, New York, 1985, Vol. 2, Chapter 5, p. 113.
[36] J. C. Fetzer, in 'Chemical Analysis of Polycyclic Aromatic Compounds', ed. T. Vo-Dinh, John Wiley, New York, 1989, Chapter 3, p. 59.
[37] F. L. Joe, J. Salemme, and T. Fazio, *J. Assoc. Off. Anal. Chem.*, 1984, **67**, 1076.
[38] M. L. Lee and B. W. Wright, *J. Chromatogr.*, 1980, **184**, 235.
[39] K. D. Bartle, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjørseth and T. Ramdahl, Marcel Dekker, New York, 1985, Vol. 2, Chapter 9, p. 193.
[40] M. L. Lee, F. J. Yang, and K. D. Bartle, 'Open Tubular Column Gas Chromatography', John Wiley, New York, 1984.

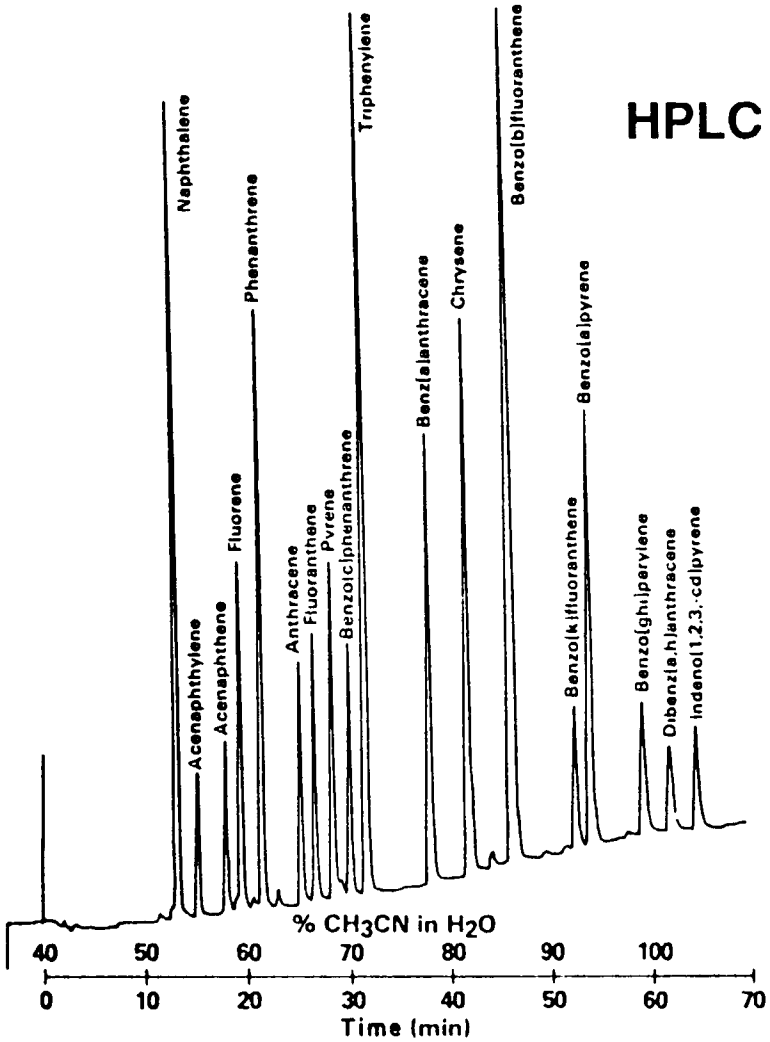


Figure 5 *Reverse phase HPLC separation of priority pollutant PAHs with detection by UV absorption*
(Reproduced with permission from S. A. Wise, W. J. Bonnett, and W. E. May, in Polycyclic Aromatic Hydrocarbons: Chemistry and Biological Effects, ed. A. Bjørseth and M. J. Dennis, Battelle Press, Columbus, Ohio, 1980)

total ion current yields a 'universal' detection chromatogram; alternatively single-ion monitoring allows focusing on one m/z value characteristic of the group of compounds under study, so that only the target isomers give peaks.

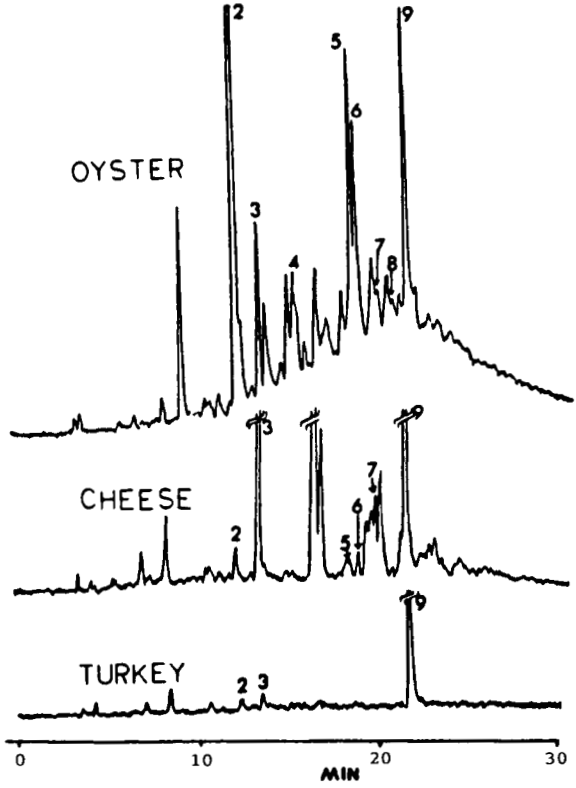


Figure 6 *HPLC chromatograms for extracts of smoked foods with fluorescence detection: 2, fluoranthene; 3, pyrene; 5, benzo[b]fluoranthene; 7, benzo[a]-pyrene; 9, benzo[b]chrysene (internal standard)*
(Reproduced with permission from F. L. Joe, J. Salemme, and T. Fazio, *J. Assoc. Offic. Anal. Chem.*, 1984, **67**, 1076)

Selected ion monitoring at m/z 202, 228, 252, 278, and 302 detects[41] pyrene and fluoranthenes, chrysene and its isomers, mono- and di-benzo-pyrenes and fluoranthenes, and the dibenzanthracene isomers. Final identification is from the full mass spectrum of a given peak – molecular weight from the molecular ion, and structural information from the fragmentation pattern, usually by means of a computer search of libraries of spectra.

Once compounds have been identified, further analyses may be made by means of retention indices – systems of reproducible retention parameters. For PAHs, a system based on the internal standards naphthalene, phenanthrene, chrysene, and picene is particularly useful. Values of the indices of 310 PAHs and their analogues have been published.[42]

[41] J. F. Lawrence and J. F. Webster, *J. Agric. Food. Chem.*, 1984, **32**, 789.
[42] D. L. Vassilaros, R. C. Kong, D. W. Later, and M. L. Lee, *J. Chromatogr.*, 1982, **252**, 1.

Quantitation in capillary GC is best achieved if sample injection is by the cold on-column method, which avoids discrimination against higher molecular weight compounds.[40]

A typical capillary GC trace with flame ionization detection of a PAH fraction from food is shown in Figure 7.

3.2.4 SFC

Although chromatography with a supercritical fluid as mobile phase was reported more than 20 years ago, the advantages of SFC have only recently been realized: SFC allows analysis of compounds of limited volatility and thermal stability at lower temperatures than in GC, but with high resolution on capillary columns.[44] While most PAHs detected in food are sufficiently stable for high-temperature GC, SFC clearly has applications for PAHs with MW > 300; the large PAHs from carbon black and coal tar[45] have been analysed by capillary SFC. The products of atmospheric oxidation or metabolic transformation discussed in Section 5 clearly represent an area of potential application of SFC.

Use of a supercritical mobile phase with a packed (HPLC) column permits[46] more rapid analysis than in HPLC mode: Figure 8 shows the SFC chromatogram of a coal-derived oil, in which the separation (within 9 minutes) of benzopyrenes and benzofluoranthenes is similar to that obtained by capillary GC of the same mixture.

3.2.5 Spectroscopic Identification

Identification in PAH analysis is also made possible from characteristic UV absorption and fluorescence spectra.[47,48] Fluorimetric detection in HPLC analyses has been recommended and chromatographic peak identities may be confirmed by using UV and fluorescence detectors in series.[37] Alternatively, the entire fluorescence spectrum of a given peak may be scanned and compared with standards[37] (for example, Figure 9). A variety of advanced spectroscopic procedures for PAHs both with and without prior separation

[43] L. Kolarovic and H. Traitler, *J. Chromatogr.*, 1982, **237**, 262.
[44] B. W. Wright and R. D. Smith, in 'Chemical Analysis of Polycyclic Aromatic Compounds', ed. T. Vo-Dinh, John Wiley, New York, 1989, Chapter 4, p. 111.
[45] R. C. Kong, S. M. Fields, W. P. Jackson, and M. L. Lee, *J. Chromatogr.*, 1984, **289**, 105.
[46] I. K. Barker, J. P. Kithinji, K. D. Bartle, A. A. Clifford, M. W. Raynor, G. F. Shilstone, and P. A. Halford-Maw, *Analyst (London)*, 1989, **114**, 41.
[47] E. L. Wehry, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjorseth, Marcel Dekker, New York, 1983, Vol. 1, p. 323.
[48] M. Zander, in 'Chemical Analysis of Polycyclic Aromatic Compounds', ed. T. Vo-Dinh, John Wiley, New York, 1989, Chapter 6, p. 171.
[49] B. P. Dunn and R. J. Armour, *Anal. Chem.*, 1980, **52**, 2027.
[50] K. Takatsuki, S. Suzuki, N. Sato, and I. Ushizawa, *J. Assoc. Off. Anal. Chem.*, 1985, **68**, 945.

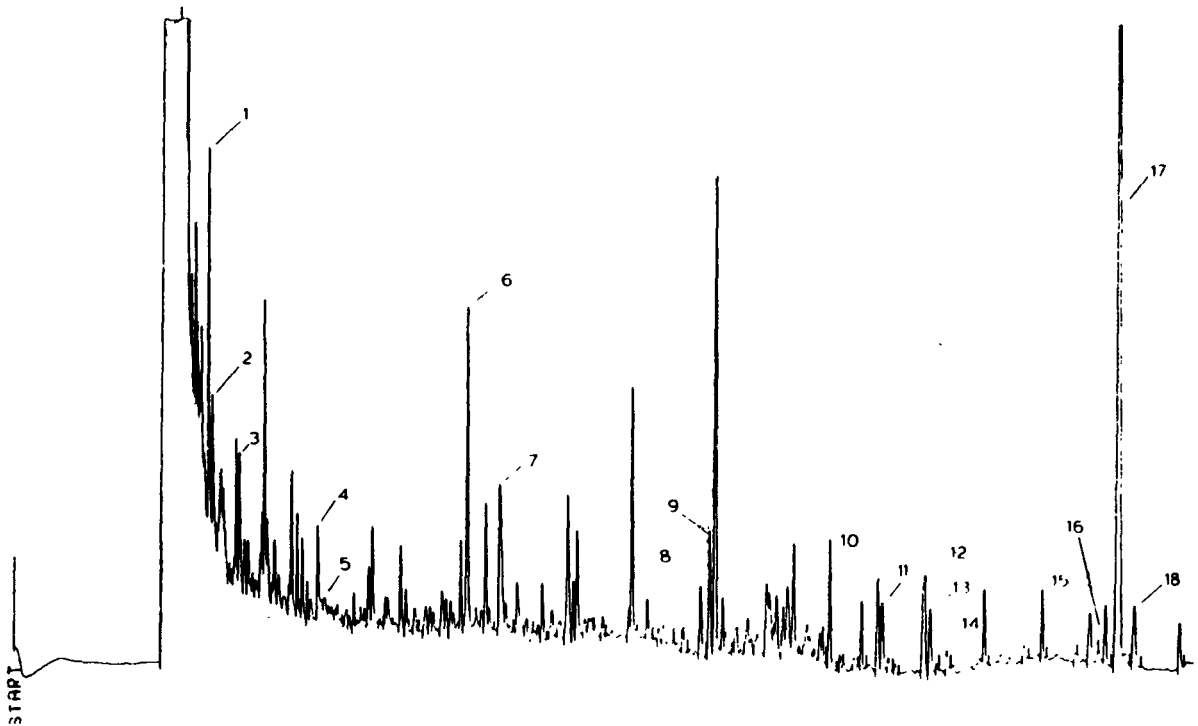


Figure 7 *Capillary GC profile of PAHs from sunflower oil with FID detection. Selected peak identification: 3, fluorene; 4, phenanthrene; 6, fluoranthene; 8, chrysene; 10, benzo[b]fluoranthene; 13, benzo[a]pyrene; 15, indeno[1,2,3-d]pyrene; 17, benzo[b]chrysene (internal standard)*
(Reproduced with permission from L. Kolarovic and H. Traitler, *J. Chromatogr.*, 1982, **237**, 262)

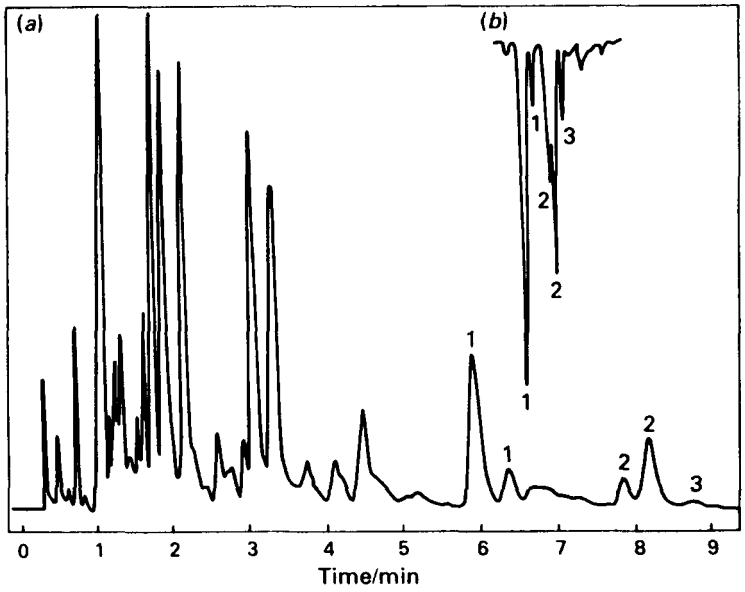


Figure 8 *Chromatograms of coal-derived PAHs,* (a) *SFC on 25* cm *ODS column, CO_2 mobile phase;* (b) *GC on 14* m *capillary column. (1), benzofluoranthenes; (2), benzopyrenes, (3) perylene*

remain to be explored in food analysis:[51] multicomponent analysis may be achieved by line-narrowing methods, and these have been complemented by new techniques involving IR, Raman, and photoionization spectroscopies.

4 Identity and Concentration of PAHs in Food

Extensive compilations of the PAH content of various foods are available.[52–55] PAHs have been reported in smoked fish and meats, grilled and roasted foods, root and leaf vegetables, vegetable oils, grains, plants, fruits, seafood, beverages, *etc.* Many early analyses were concerned only with the determination of the well-known carcinogen benzo[*a*]pyrene and a recommended TLC method for screening for this compound has been proposed;[56] more recently the high-resolution methods discussed in Section 3 have been applied across a wider range of PAHs. These analyses extend only to *ca.* 10–20 compounds, however, and alkylated compounds are seldom reported.

[51] 'Chemical Analysis of Polycyclic Aromatic Compounds', ed. T. Vo-Dinh, John Wiley, New York, 1989.
[52] J. W. Howard and T. Fazio, *J. Assoc. Off. Anal. Chem.*, 1980, **63**, 1077.
[53] M. T. Lo and E. Sandi, *Residue Rev.*, 1978, **69**, 35.
[54] J. Santodonato, P. Howard, and D. Basu, *J. Environ. Path. Toxicol.*, 1981, **5**, 1.
[55] M. S. Zedeck, *J. Environ. Path. Toxicol.*, 1980, **3**, 537.
[56] G. Grimmer and J. Jacob, *Pure Appl. Chem.*, 1987, **59**, 1735.

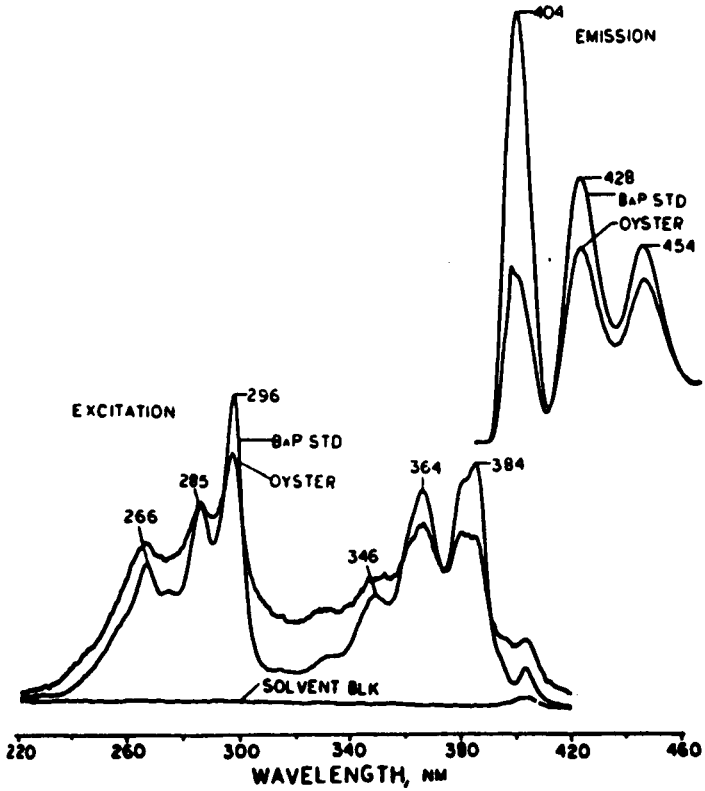


Figure 9 *Excitation and emission spectra of standard benzo[a]pyrene and a component with the same retention time isolated from HPLC analysis of an oyster extract*
(Reproduced with permission from F. L. Joe, J. Salemme, and T. Fazio, *J. Assoc. Offic. Anal. Chem.*, 1984, **67**, 1076)

Examples of analytical results for smoked (Table 4) and unsmoked (Table 5) products and for meat cooked in different ways (Table 6) are given here. There is general consistency[22,57] among results for similar sample types, usually at the 1–10 $\mu g\,kg^{-1}$ (p.p.b.) level, but occasionally higher in smoked or grossly contaminated foods; it is nonetheless true that both intra- and inter-laboratory tests on identical materials have shown intermediate or poor precision.[58-60] Capillary GC with MS detection has been suggested[61] as the most reliable procedure because of the superior resolving power and detection specificity.

[57] M. J. Dennis, R. C. Massey, D. J. McWeeny, and D. H. Watson, in 'Polynuclear Aromatic Hydrocarbons', ed. M. W. Cooke and M. J. Dennis, Battelle Press, Columbus, Ohio, 1982, p. 405.
[58] J. F. Lawrence and D. F. Weber, *J. Agric. Food Chem.*, 1984, **32**, 794.
[59] G. Grimmer and J. Jacob, *Pure Appl. Chem.*, 1987, **59**, 1729.
[60] J. F. Uthe and C. J. Musial, *J. Assoc. Off. Anal. Chem.*, 1988, **71**, 363.
[61] K. D. Bartle, M. L. Lee, and S. A. Wise, *Chem. Soc. Rev.*, 1981, **10**, 113.

Table 4 *Levels[a] of PAHs in smoked food* ($\mu g\ kg^{-1}$)

	Cheese	*Ham*	*Bacon*
Fluoranthene	3.5	1.2	1.1
Pyrene	7.0	1.7	1.1
Benz[*a*]anthracene	0.8	0.6	0.4
Benzo[*b*]fluoranthene	0.4	0.2	0.2
Benzo[*a*]pyrene	0.5	0.2	0.1
Benzo[*ghi*]perylene	0.4	0.1	ND
Dibenz[*a,h*]anthracene	0.1	ND	ND

[a] From F. L. Joe, J. Salemme, and T. Fazio *J. Assoc. Offic. Anal. Chem.*, 1984, **67**, 1076. ND: not determined.

Table 5 *Average content[a] of some PAHs in foods* ($\mu g\ kg^{-1}$)

	Cereals	*Meat*	*Fish*	*Oils and fats*	*Vegetables*
Fluoranthene	1.4	0.5	0.8	1.8	1.5
Pyrene	1.9	0.6	0.8	2.8	1.2
Benz[*a*]anthracene	0.4	0.05	0.1	1.0	0.2
Chrysene	0.8	0.2	0.7	1.2	0.9
Benzo[*b*]fluoranthene	0.2	0.05	0.1	0.9	0.2
Benzo[*a*]pyrene	0.3	0.05	0.1	1.6	0.1
Benzo[*ghi*]perylene	0.3	0.05	0.1	1.3	0.1
Dibenz[*a,h*]anthracene	0.05	0.01	0.03	0.05	0.01

[a] From M. J. Dennis, R. C. Massey, D. J. McWeeny, M. E. Knowles, and D. Watson, *Food Chem. Toxicol.*, 1983, **21**, 569.

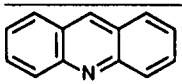
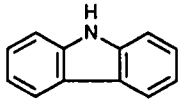
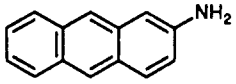
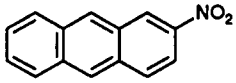
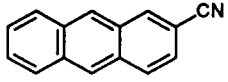
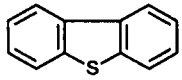
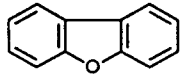
Table 6 *Levels[a] of PAHs in differently cooked meat* ($\mu g\ kg^{-1}$)

	Charcoal-broiled steaks	*Barbecued ribs*
Fluoranthene	43	49
Pyrene	0.9	1.4
Benz[*a*]anthracene	1.4	3.6
Chrysene	0.6	2.2
Benzo[*a*]pyrene	5.8	10.5
Benzo[*ghi*]perylene	6.7	4.7

[a] From T. Fazio and J. W. Howard, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjørseth, Vol. 1, Marcel Dekker, New York, 1983, p. 496.

In addition to PAHs, the presence of an enormous number of heterocyclic analogues is possible in food, usually arising from environmental contamination from fossil-fuel sources. These include polycyclic aromatic nitrogen heterocycles and their sulphur and oxygen isosteres (Table 7). Various schemes have been devised for the concentration and separation of hetero-compounds, usually employing column chromatography followed by

Table 7 *Classification of PAHs*

Structure	*Name*	*Class*
	Acridine	Polycyclic aromatic nitrogen heterocycles (PANH)
	Carbazole	
	2-Aminoanthracene	Amino polycyclic aromatic Hydrocarbons (APAH)
	2-Nitroanthracene	Nitro polycyclic aromatic hydrocarbons (NPAH)
	2-Cyanoanthracene	Cyano polycyclic aromatic hydrocarbons (CPAH)
	Dibenzothiophene	Polycyclic aromatic sulphur heterocycles (PASH)
	Dibenzofuran	Polycyclic aromatic oxygen heterocycles (PAOH)

capillary GC with selective detection[62,63] (flame photometry for PASHs and thermionic detection for PANHs). Although gross contamination with PASHs of fish from a coal-product polluted river has been demonstrated by such methods,[63] a simpler analytical scheme based on HPLC with fluorescence detection suggested[64] that PANHs were not present at levels ≥5 p.p.b. in salmon.

The formation of highly carcinogenic NPAHs in smoked foods by reaction of PAHs with nitrogen oxides during the smoking process has

[62] D. W. Later, M. L. Lee, K. D. Bartle, R. C. Kong, and D. L. Vassilaros, *Anal. Chem.*, 1981, **53**, 1612.
[63] D. L. Vassilaros, P. W. Stoker, G. M. Booth, and M. L. Lee, *Anal. Chem.*, 1982, **54**, 106.
[64] F. L. Joe, J. Salemme, and T. Fazio, *J. Assoc. Off. Anal. Chem.*, 1986, **69**, 218.

been postulated;[65] alternatively, fruit and vegetables originating from sites exposed to road traffic could also be contaminated with the NPAHs known to be present in diesel emissions.[66] In fact, only three of 27 samples analysed contained detectable levels of NPAHs, and these were all foods consumed indirectly.

5 Significance of Contamination of Food by PAHs

While there is little firm epidemiological evidence for the risk of PAHs from food, dietary intake has been identified as the principal route for exposure to PAHs for non-smokers. 'Normal' diets contain[22,67,68] significant concentrations of a variety of PAHs, as is clearly shown by Table 5. Some smoked foods, contaminated shellfish, *etc.* contain larger concentrations, but these are less often consumed. In fact, the concentrations in Table 5, when multiplied by the weight of the food in the average diet, lead[22] to amounts which are of the same order of magnitude as those in the smoke from *ca.* 20 high-tar cigarettes[69] and even more low-tar cigarettes (Table 8).

Although it can be argued that the ingestion routes are insufficiently similar to permit comparisons, ingestion of PAH is a complex process – a significant proportion of inhaled PAHs are removed from the lung into the

Table 8 *Daily intake (μg) of PAHs from food and tobacco smoke*

	From food and beverages[a]	*From 20 cigarettes*[b]
Fluoranthene	1.0	1.7
Pyrene	1.1	1.4
Benz[*a*]anthracene	0.2	0.5
Chrysene	0.5	0.1
Benzo[*b*]fluoranthene	0.2	0.1
Benzo[*a*]pyrene	0.3	0.3
Benzo[*ghi*]perylene	0.2	0.1
Dibenz[*a,h*]anthracene	0.03	0.1

[a] From M. J. Dennis, R. C. Massey, D. J. McWeeny, M. E. Knowles, and D. Watson, *Food Chem. Toxicol.*, 1983, **21**, 569.
[b] From M. L. Lee, M. Novotny, and K. D. Bartle, *Anal. Chem.*, 1976, **48**, 405.

[65] M. J. Dennis, G. C. Cripps, I. Venn, R. C. Massey, D. J. McWeeny, and M. E. Knowles, in 'Polycyclic Aromatic Hydrocarbons', ed. A. Bjørseth and M. J. Dennis, Battelle Press, Columbus, Ohio, 1983, p. 229.
[66] R. Nakagawa, *Mutat. Res.*, 1983, **124**, 201.
[67] J. P. Tuominen, H. S. Pysala, and M. Sauri, *J. Agric. Food. Chem.*, 1988, **36**, 118.
[68] L. Shuker and B. G. Bennet, 'Exposure Commitment Assessments of Environmental Pollutants', Monitoring and Assessment Research Centre, University of London, Technical Report, 1988, Vol. 6.
[69] M. L. Lee, M. Novotny, and K. D. Bartle, *Anal. Chem.*, 1976, **48**, 405.

gastrointestinal tract by mucociliary clearance and swallowing, so that the doses in Table 8 may well be comparable. These involuntary dose levels from generally consumed foods would appear to be high enough to cause disquiet, and should cause at least similar concern to that over the perceived health hazards of diesel particulate emissions,[70] in which PAHs are strongly implicated. The major origin of dietary PAHs in the collection of combustion-generated pollutants by grain fields is not proven, but clearly merits further investigation. Increasing industrial development worldwide is likely to lead to large increases in anthropogenic emissions and further accumulation of PAHs.

Whereas the relatively high concentrations of PAHs in smoked and barbecued foods have, in the past, led to the targeting of such materials as health risks, it now seems more likely that cereals,[22,67] especially in the form of flour, are a greater hazard.

PAHs are thermally stable products of numerous combustion reactions, but they are nonetheless chemically reactive, particularly towards oxidants such as ozone and singlet molecular oxygen, and with hydroxyl radicals and oxides of nitrogen and sulphur.[71] These reactions have important implications for analytical procedures, especially during extraction and clean-up and the exposure of PAHs on TLC plates to ozone; reactions of anthracene, benzo[*a*]pyrene, and benz[*a*]anthracene are particularly rapid with half lives of 0.15, 0.58, and 1.35 hours respectively.[72]

Atmospheric reactions of PAHs could also significantly influence the health risks from PAHs in food. Nitration of PAHs to form NPAHs which are potent direct acting mutagens has been demonstrated;[71] such compounds are known atmospheric[73] and engine exhaust[66] constituents and therefore may be expected to be collected on crops. However, as discussed above, Dennis *et al.* found[65] detectable levels of NPAHs in only a few foods, none of which is consumed directly.

Of greater concern is the so-far uninvestigated possibility that atmospheric oxidation products of PAHs may be similar to those produced by *in vivo* metabolic activation, and that hence more potent carcinogens could be deposited on crops and find their way into food by routes similar to those followed by the PAH burden. Thus, the highly mutagenic 4,5-epoxide of benzo[*a*]pyrene has been detected[74] in airborne particulates, and shown to originate by ozonolysis of the parent. Reaction with singlet oxygen yields quinones of anthracene and benzo[*a*]pyrene, the latter being converted into the mutagenic 1,6-, 3,6-, and 6,12-diones, while direct

[70] M. P. Walsh, S. A. E. Technical Paper Series No. 871072, Washington, DC, 1987.
[71] K. A. Van Cauwenberghe, in 'Handbook of Polycyclic Aromatic Hydrocarbons', ed. A. Bjorseth and T. Ramdahl, Marcel Dekker, New York, 1985, Volume 2, Chapter 10, p. 351.
[72] M. Katz, C. Chan, H. Tosine, and T. Sakuma, in 'Polynuclear Aromatic Hydrocarbons: Third International Symposium on Chemistry and Biology', ed. R. W. Jones and P. Leber, Ann Arbor Science Publishers, Ann Arbor, Michigan, 1979, p. 171.
[73] T. Nielson, B. Seitz, and T. Ramdahl, *Atmos. Environ.*, 1984, **18**, 2159.
[74] J. N. Pitts, D. M. Lokensgard, P. S. Ripley, K. A. Van Cauwenberghe, L. Van Vaeck, S. D. Schaffer, A. J. Thill, and W. L. Belser, *Science*, 1980, **210**, 1347.

conversion of chrysene to mutagenic derivatives has also been shown.[75] Many carbonyl derivatives of aromatics are known to play an important role in human biochemistry.

[75] D. A. Lane, in 'Chemical Analysis of Polycyclic Aromatic Compounds', ed. T. Vo-Dinh, John Wiley, New York, 1989, Chapter 2, p. 31.

CHAPTER 4

Food Production Contaminants: Control and Surveillance

JONATHAN R. BELL and DAVID H. WATSON

1 Introduction

The term food production contaminants can be used to describe a wide variety of chemicals that might be present in food, such as residues of pesticides and veterinary drugs, chemicals migrating into food from packaging, certain naturally occurring toxicants, and adventitious chemical contaminants from food manufacture. However, as other chapters in this book describe work on agrochemical residues and food packaging migrants, special emphasis will be given to these groups here.

The processes of control and food chemical surveillance should be closely linked, whether for food production contaminants or the several other groups of chemicals that are found in food. Where both processes are well developed, as in the UK and a number of other developed countries, control of food production contaminants, or indeed any other chemical contaminants in food, draws upon food chemical surveillance as a source of information in a number of ways. These include identifying a control need, providing the necessary scientific detail in support of a control mechanism that is under development, and checking on the effectiveness of controls once in place.

For controls to be effective, therefore, food chemical surveillance must provide a well organized, reliable source of information. In the UK the mechanism that has developed to provide central Government with such information draws on an extensive body of analytical results. Many thousands of samples of food are analysed every year, mainly by chemical techniques, such as gas chromatography, high performance liquid chromatography, and mass spectrometry. This level of technology is essential since the amounts of contaminants present are generally in the region of parts per million or less. For this reason, and because food is a very complex

matrix for the analyst, carefully developed methods of extracting the contaminants from food are essential.

In developing such extraction methods it is important for the analyst:

To take full account of the role that the resulting data will play in aiding the control process;
To ensure that the results have been subjected to an adequate quality assurance procedure;
To devise automated procedures wherever possible; and
To consider information that is already available in deciding what work needs to be done.

The UK food chemical surveillance programme is co-ordinated by the Steering Group on Food Surveillance (SGFS),† a senior UK Government advisory committee. The role and *modus operandi* of this Group have been stated as follows:[1]

'1. The Steering Group (on Food Surveillance)'s function is to identify and evaluate problems, and to propose practical solutions taking into account legal and other constraints.
2. Wherever possible the Steering Group should anticipate problems and act to prevent them.
3. Resources should be used in a cost effective fashion.
4. The Steering Group should publish its intended programmes of work and their results.
5. Special attention should be given to the surveillance of foods consumed by specific groups of people whose diet might put them at more risk than the "average" consumer.
6. Surveillance of emergent foods* and of foodstuffs produced by novel methods should be carried out, once their use is established.
7. Working parties reporting to the Steering Group should actively seek close co-operation with those bodies responsible for environmental surveillance, as and when appropriate.
8. The criteria by which the effectiveness of the surveillance programme of the Steering Group on Food Surveillance may be assessed, are as follows:

8.1 The purity and nutritional quality of the UK food supply should be sustained and enhanced. Contamination of food should be reduced to the lowest practicable level. The highest priority should be given to those contaminants that are believed to be harmful. The food intake of the population should be nutritious.

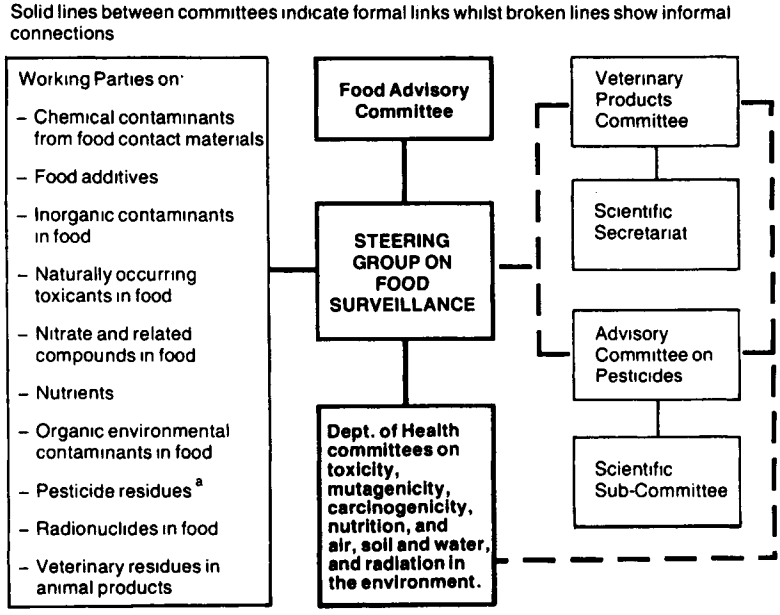
[1] Steering Group on Food Surveillance, 'Food Surveillance 1985 to 1988', Food Surveillance Paper No. 24, HMSO, London, 1988.

†Recently renamed the Steering Group on Chemical Aspects of Food Surveillance.

*Foods that were not previously commonly consumed, for example new varieties of vegetables.

8.2 The confidence of the public and scientists in food surveillance should be increasing.'

The SGFS provides guidance to ten working parties which develop and co-ordinate surveillance work in their specific areas (Figure 1). The programmes of these working parties consist largely of surveys and related research, much of which is on the development of analytical methods. The results of this work are not only considered by the SGFS but by the other Government senior advisory committees shown in Figure 1. The interests of these committees cover the full breadth of issues relevant to the work of the SGFS. The Veterinary Products Committee (VPC) advises Ministers on the licensing of veterinary drugs. The Advisory Committee on Pesticides (ACP) advises Ministers on any matter relating to the control of pests, including which pesticides may be approved for use in the UK. Clearly it is important, in this context, that the results of analytical surveys of drug or pesticide residues, as well as the toxicological and other health-related aspects of these surveillance data, are fully explored by these two committees. The Food Advisory Committee, also referred to in Figure 1, provides expert advice on all food safety issues associated with the chemical contamination of food. As this committee has no extensive toxicological expertise of its own it seeks advice on health-related issues from the Department of Health's Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment.



Note: a: This Working Party reports to both the Steering Group on Food Surveillance and the Advisory Committee on Pesticides.

Figure 1 *The Steering Group in relation to other committees*

The considerable body of information, intelligence, and judgement that derives from the food surveillance system is published in a series of reports.[2] Figure 2 shows a selection of these. As well as providing a source of data, and comment and advice from the expert committees on specific issues, these Food Surveillance papers also chart the growth of the programme of work over the past decade or so. Central to this growth has been the development of work on food production contaminants discussed in the latter part of this chapter. However, to set this work in context it is necessary to consider the system of control applied to these substances in the UK.

Figure 2 *Food Surveillance Paper covers*

2 Control of Food Production Contaminants in the UK

Legislative control over residues and food contact materials is mainly derived from the powers contained in three Acts of Parliament:

For *pesticide residues*: control derives from the Food and Environment Protection Act 1985. Part III of this Act provides powers to set maximum levels for pesticides. Specific power to control pesticide residues is provided by Clause 16(2)(k) which says that 'Ministers

[2] Steering Group on Food Surveillance, Food Surveillance Papers Nos. 1 to 30, HMSO, London, 1978–90.

may.... by regulations... specify how much pesticide or pesticide residue may be left in any crop, food or feeding stuff....'.

For *veterinary drug residues*: the Medicines Act 1968 defines safety, quality, and efficacy as the criteria to be considered in licensing veterinary medicines. In this context safety includes that of consumers as well as those using or receiving the medicines and environmental safety.

For *food contact materials*: control is exercised under the European Communities Act 1972. As this implies, controls derive from European Community legislation to which the UK Government, supported by industry, has made and continues to make a major contribution.

Legislative powers deriving from the first two Acts provide a statutory basis for the advice provided to Government on the licensing of veterinary products and the approval of pesticides by the VPC and ACP respectively. All products must be approved before they can be sold and these Committees consider applications for approval and advise Ministers of their views. In reaching their conclusions both committees take into account the levels of residues likely to remain in the product after treatment. Thus control on residues of pesticides and veterinary medicines in this country is exercised through a pre-approval system for the precursor chemicals. Food chemical surveillance provides an important source of information about the effectiveness of these controls in restricting the resulting levels and incidences of residues in the food supply. It also provides a check on whether the products are being used as approved.

This approach to controlling residues is mirrored in many other countries, such as the USA and Canada, and many of the UK's partners in the European Community. Harmonization of controls on pesticide and veterinary drug residues, and perhaps to a lesser extent on food contact materials, is now becoming a major activity worldwide. Much of the scientific work on the safety of the substances involved is carried out under the aegis of the World Health Organization and the Food and Agriculture Organization, through the respective committees of the Codex Alimentarius Commission. Their published recommendations (for example in reference 3) have an international influence on the control of food production contaminants. They are often taken into account by governments when setting national controls and when agreeing international controls such as those applying within the European Community.

Although legislation provides an important basis for the control of many food production contaminants, it is sometimes desirable to use other methods in place of or in addition to this. The options include:

Collaborative research to resolve problems. A technological solution to a problem can be very effective in the right circumstances. Collaboration,

[3] Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, 'Guide to Codex Recommendations Concerning Pesticide Residues, Part 7. Codex Guidelines on Good Practice in Pesticide Residue Analysis', Document CAC/PR7-1984, FAO/WHO, Rome, 1984.

for example between scientific experts involved with control and industrial production, can help to resolve problems which have a high technical content without the need for legislation.

Codes of practice are effective where a responsible industry can overcome a problem by applying rules of good practice.

Advice to consumers by Government that is clear, soundly based, and well communicated can provide an effective means of reducing exposure to chemical contaminants in food, particularly where they are associated with processing of food in the home.

The choice of which approach to follow depends on a variety of factors. But most important of all is the need to ensure consumer protection and choice.

The consumer is the intended beneficiary of any action taken to control the presence of chemicals in food and it is important to inform him or her about the measures that are being implemented in their names. An example of this in the UK is the recent publication of a booklet about pesticides and food. This booklet entitled 'Pesticides and Food: A Balanced View' is available free on request. It explains in straightforward language the basis for pesticide usage and the controls on this and on pesticides residues in the UK. This is one of a series of booklets on food safety issues now available (from Food Sense, London SE99 7TT). The series also includes the booklet 'Food Surveillance' which provides the public with the types of information discussed in this chapter.

3 Surveillance of Food Production Contaminants in the UK

The Government food chemical surveillance system described earlier is used extensively to study migrants from food contact materials and residues of pesticides and veterinary drugs. Although approaches used in each case differ in some details the net result is the same, namely the generation of data on the levels and likely intakes of these from food. Intake data, in particular, provide a common core to much of the surveillance work on chemical contaminants in food.

The value of assessing intakes is illustrated by some recently reported work of the Working Party on Pesticide Residues. This working party used surveillance data to estimate the average intakes of a wide variety of pesticides.[4] The resulting information (Table 1) showed that the intakes were very low and, where comparison with previous information was possible, that they had decreased in some cases. This exercise also allowed comparison with Acceptable Daily Intakes (ADIs) established by national and international scientific bodies. The ADI is a toxicological term which has been defined[4] as 'the amount of a chemical which can be consumed

[4] Steering Group on Food Surveillance, 'Report of the Working Party on Pesticide Residues: 1985–88', Food Surveillance Paper No. 25, HMSO, London, 1989.

Table 1 *Computed average intakes and Acceptable Daily Intakes for pesticide residues found in total diet studies in 1981 and 1984–85*

	Intakes (mg per person per day)		
Pesticide	*1981*	*1984–85*	*Acceptable Daily Intake*
Total DDT	0.0020	0.0005	1.4
Dieldrin	0.0007	0.0005	0.007
γ-HCH	0.0020	0.0005	0.7
Chlorpyrifos	NC	0.0001	0.7
Malathion	NC	0.0001	1.4
Pirimiphos-methyl	NC	0.0018	0.7
Cypermethrin	NC	<0.0001	3.5
Permethrin	NC	0.0001	3.5
Quintozene	<0.0004	0.0002	0.49
Tecnazene	0.0029	0.0043	0.7

Note: NC: Not calculated since no residues were detected in the 1981 total diet study.

every day for an individual's entire lifetime in the practical certainty, on the basis of all the known facts, that no harm will result'. This potentially powerful measure provides a quantitative value with which to compare data derived from food chemical surveillance. In the case of the information in Table 1 the comparisons for each pesticide demonstrated that intakes were considerably lower than the respective ADIs. The use of this approach to safety assessment is less widespread for veterinary drug residues and chemicals migrating from food contact materials since fewer ADIs are available. Although there is a growing acceptance of the need to assess these food production contaminants in this way, this is heavily dependent on the development of the necessary body of toxicological information from which ADIs can be derived.

Where ADIs are not available, food chemical surveillance must rely upon other measures of hazard to identify where problems may exist. Sometimes the detection alone of a production contaminant in food identifies a problem. This is the case where presence of the contaminant indicates illegal usage of a pesticide or veterinary drug. The following example, from UK work on veterinary drug residues,[5] illustrates this and also demonstrates the value of transmitting food chemical surveillance information to enforcement authorities. Figure 3 summarizes the surveillance results for residues of a group of synthetic hormone growth promoters – the stilbenes – in samples from cattle, calves, pigs, and sheep in Great Britain from 1981 to 1988. This work was started purely as a surveillance exercise – to quantify the levels and incidences of one stilbene compound, diethylstilboestrol, which was licensed for use as a pig feed

[5] Steering Group on Food Surveillance, 'Anabolic, Anthelmintic and Antimicrobial Agents', Food Surveillance Paper No. 22, HMSO, London, 1987.

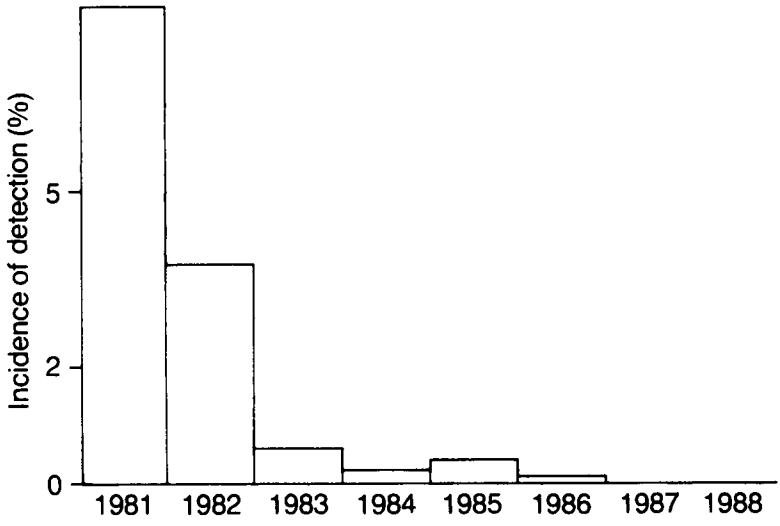


Figure 3 *Stilbene residues. The use of stilbene growth-promoting hormones in food producing animals such as cattle, calves, pigs and sheep was prohibited in 1982. Those found guilty of their illegal use have been fined. As the graph shows, a marked decrease in the incidence of residues has been measured.*

additive. However, fairly soon after the work had been started the use of this and other stilbene compounds in food-producing animals was prohibited in the UK. Although the incidence of residues declined rapidly, some evidence of the continuing use of this hormone was found. Following further investigation, two prosecutions resulted. By 1987 there was no evidence of further usage.

In general, however, food production contaminants are present in food as the result of legal usage and in the great majority of cases the levels found do not indicate a problem. Nevertheless it is prudent to reduce the levels of food production contaminants as far as possible.

Whereas the study of residues of pesticides and veterinary drugs via surveillance is largely based on the analysis of food samples drawn from the general food supply, much of the surveillance work on food contact materials in the UK has involved the study of model systems in the laboratory. In this way interference from similar environmental contaminants to those arising from the materials in question can be taken into account. Although this approach is now being increasingly supplemented with the surveillance methods used in work on residues, there is still value in continuing to use model systems as well. An example of this approach was the recently reported[6] work on migration of the plasticizer DEHA [di-(2-ethylhexyl) adipate], from PVC cling film into test pieces of cheddar cheese. Figure 4 summarizes the kinetics of this migration.

[6] Steering Group on Food Surveillance, 'Survey of Plasticiser Levels in Food Contact Materials and in Foods', Food Surveillance Paper No. 21, HMSO, London, 1987.

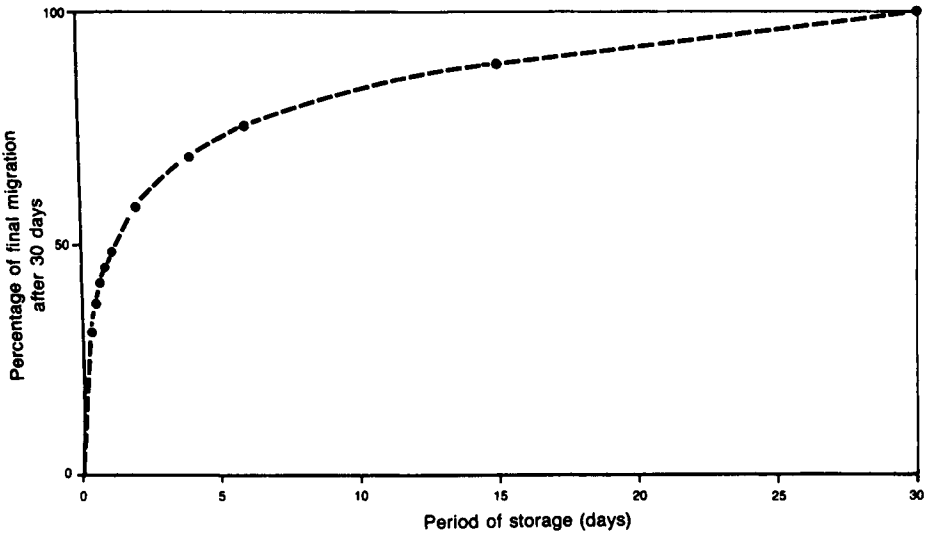


Figure 4 *Migration of DEHA into cheddar cheese at 5 °C*

The surveillance of production contaminants in the food chain is a growing area of science. The numbers of chemicals that could be searched for is large, probably in excess of a thousand. Although there would be little or no point in carrying out surveillance for some of these, such as herbicides that would kill crops rather than leave residues, the remaining list of compounds is still large. Food surveillance, both in the UK and overseas, is probably furthest advanced for pesticide residues. The reasons for this are largely historical as the international scientific community first became interested in pesticide residues in the early 1960s, so that a very extensive database now exists. Many of the lessons learnt in the development of surveillance techniques for pesticide residues have been applied in work on other food production contaminants and indeed other areas of food surveillance.

The development of the UK surveillance programme to give adequate coverage of the large number of chemicals involved and the great variety of foods consumed has necessitated the setting of priorities. An example of this is shown in Figure 5. This is a list of recent projects undertaken by the Working Party on Pesticide Residues. The list ranges from the very specific, such as work on one pesticide, dinoseb, to the very broad such as monitoring of staple items of the diet – bread, milk, and potatoes. These projects were identified as being of high priority based on scientific experience and knowledge obtained from, for example, international fora and the scientific literature.[4] As the potential for pesticide residues to occur can vary according to the commodity, the country of origin, and the type of pest[4] it is essential that care is taken to ensure that the resources available are focused on those areas where residues are most likely to be

Project	Start	Finish
Pesticide residues in eels	1986	1988
Pesticide residues in barley	1987	1987
Pesticide residues in wine	1987	
Monitoring of dietary staples: bread, milk and potatoes	1987	
Residues of dinoseb in UK crops	1987	1989
Pesticide residues in animal feeding stuffs	1987	
Organochlorine residues in venison	1987	1989
Pesticide residues in infant foods	1987	
Trial to determine DDT levels in brassicas	1987	
Monitoring of residues in fruit and vegetables	1988	
Monitoring of residues in cereals and cereal products	1988	
Monitoring of residues in animal products	1988	

Figure 5 *Extract from the programme of the Working Party on Pesticide Residues*

present. The alternative of sampling every type of foodstuff for every possible pesticide residue is impractical and would be very wasteful. Although fewer in number, veterinary drugs should be studied in a similar way for the same reasons. Different factors influence the surveillance of migrants from food contact materials since it is possible to carry out considerable laboratory work on model systems. This in itself can act as a guide to the setting of priorities, as well as contributing data to the overall work programme.[7]

4 Discussion

Objective information about residues *etc.* must provide the key to establishing consumer confidence in the sensible use of chemicals in the production of food and every effort should be made to communicate relevant scientific information both in the form of technical documents intended for the specialist, such as Food Surveillance papers,[2] and in material for a more general audience.

At the present time the degree of hazard arising from chemicals in food is perceived differently by the public and scientists. Figure 6 summarizes the views of some scientists on the ranking of risk from food production contaminants and two other groups of food chemicals. Figure 7 provides a very approximate assessment of public perception of the same chemicals. The duty of scientists is not only vigorously to review data on food production contaminants, but also to communicate their findings as widely as possible. Recipients of such information should include not only the

[7] Steering Group on Food Surveillance, 'Migration of Substances from Food Contact Materials into Food', Food Surveillance Paper No. 26, HMSO, London, 1989.

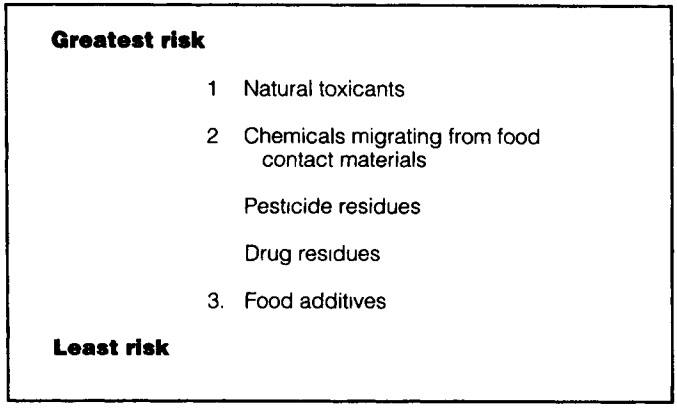


Figure 6 *Some scientists' perception of food chemicals*

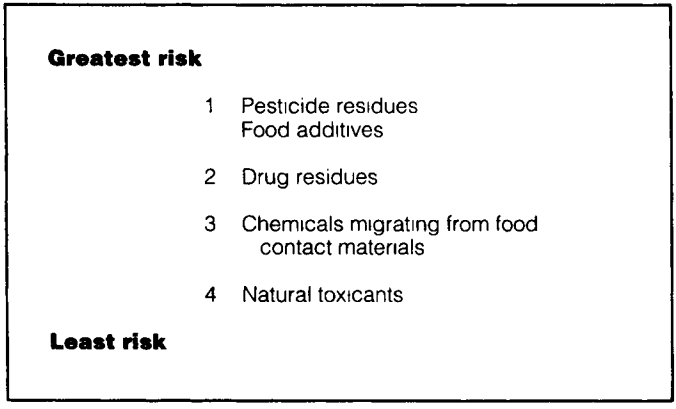


Figure 7 *Approximate assessment of public perception of food chemicals*

general public but the media, which in recent times have used information about food production contaminants to raise the public's level of concern about the safety of the foods they eat. It is important to place any hazard in perspective and to direct attention away from substances of little or no risk. In this regard more work needs to be done on assessing the health implications of substances such as naturally occurring toxicants in food about which little is currently known. By quantifying the risk from compounds such as these, the relative risks from dietary exposure to food production contaminants such as drug or pesticide residues or food packaging migrants can be better assessed.

5 Summary

Food production contaminants as a class include a wide variety of chemicals that might be present in food. Three of the more important

groups are: pesticide residues, veterinary drug residues, and chemical migrants from food contact materials. The processes of control and surveillance for these chemicals are closely linked. In the UK food surveillance for these and other contaminants is co-ordinated for central Government by the Steering Group on Food Surveillance. The relationships between this interdepartmental committee and other senior advisory committees are described together with their relevant functions.

In the UK, control of food production contaminants is provided through legislation, the co-operation of industry, and government advice to the consumer. Similar means of control are employed in other developed countries, but action on a worldwide basis is essential if contaminants in food are to be properly controlled.

To be effective, surveillance for food production contaminants requires the use of sophisticated analytical methodology, realistic priority setting, and careful interpretation of the resulting data. The use of surveillance data to calculate the intake of chemicals from the diet and the comparison of the results so obtained with Acceptable Daily Intakes, where these exist, has helped to improve assessments of the risk to health posed by these substances.

Scientific work on food production contaminants has an important role to play in informing the public debate about the safety of the food supply. By making the objective information obtained in this way widely available, both specialists and the general public can begin to put the various risks associated with the ingestion of these chemicals into perspective.

CHAPTER 5

Toxicology and Regulatory Control of Components of Food Contact Plastics

RUPERT PURCHASE

1 Introduction

1.1 Food Contact Materials

Although some of the materials traditionally used for packaging and distributing food[1] (paper, cellophane, metals, ceramics, glass, rubber, wood, and fabric) are not without toxicity, the adoption of plastic materials for containing and processing food has presented a major problem for toxicologists, legislators, and analytical chemists for at least three decades.

The stimulus for analytical chemists to identify and quantify the components of plastics (particularly plasticizers) which have migrated into food is discussed in Chapter 6. In this chapter, the legislative problems posed by food contact plastics are described, and some of the toxicological issues arising from their use are discussed.

1.2 Plastics Used for Food Contact Materials

Two types of plastic are used for food contact materials: *thermosets*[2] (plastics which undergo an irreversible change on heating and do not soften[1]), *e.g.* phenol–formaldehyde, urea–formaldehyde, and *thermoplastics*[1–3] (plastics which can be softened by heating and hardened on cooling, repeatedly, provided no chemical decomposition occurs), *e.g.* low density polyethylene – which accounts for the biggest proportion of plastics used in

[1] N. T. Crosby, 'Food Packaging Materials', Applied Science Publishers, Barking, 1981.
[2] F. A. Paine and H. Y. Paine, 'A Handbook of Food Packaging', Leonard Hill (Blackie Group), Glasgow, 1983.
[3] J. H. Briston, in 'Developments in Food Packaging-1', ed. S. J. Palling, Applied Science, London, 1980, Chapter 2.

food packaging, high density polyethylene, polypropylene, poly(vinyl chloride) (PVC), and poly(vinylidene chloride) copolymer (VDC).

High molecular weight polymers themselves have a limited solubility in aqueous and fatty systems and present little toxic hazard. However, the properties of polymers – their resistance to heat and light, flexibility, stability on storage – can be drastically altered by the incorporation of additives.[4]

Concern over the safety-in-use of food contact plastics arises principally from the possible toxicity of low molecular weight and structurally diverse constituents that may be inadvertently or deliberately present in the polymer and leached into food during storage.[1]

Two types of low molecular weight product may be present:[1,4]

(i) Polymerization residues including monomers and oligomers (molecular weight up to 200), polymerization aids (*e.g.* catalysts, cross-linking agents, inhibitors), solvents, emulsifiers and wetting agents, raw material impurities, plant contaminants, decomposition, and side-reaction products.

(ii) Additives and processing aids, *e.g.* antioxidants, antiblocking agents, antistatic agents, heat and light stabilizers, plasticizers, lubricant and slip agents, pigments, fillers, mould release agents, and fungicides.

2 Regulatory Control of the Components of Food Contact Materials

2.1 European Approaches[5]

A number of member states of the European Community (EC) have their own legislation or guidelines based on positive lists of monomers, additives, and other ingredients. These lists may also recommend compositional limits, *i.e.* the maximum amount of each component which may be present in a material. Some countries have also imposed either a specific or a global migration limit on plastic components. For global migration it is generally recommended that plastic materials shall not transfer their constituents to food in levels exceeding either 10 mg dm^{-2} of surface area of the food contact material or 60 mg kg^{-1} of foodstuff (if it is impracticable to estimate the surface area which is in contact with food).

2.2 The UK Approach – The BIBRA/British Plastics Federation Code of Practice

Current regulatory control in the UK requires that materials intended to come into contact with food shall be manufactured such that the components of those materials are not transferred to foods in quantities which could endanger human health or bring about a deterioration in the quality

[4] 'Plastics Additives Handbook', ed. R. Gächter and H. Müller, Hanser Publishers, Munich, 1987.

[5] R. Leimgruber, in ref. 4, chapter 18, p.685.

of the foodstuffs [Statutory Instrument (SI) 1987 No. 1523]. Hitherto the UK has not introduced a strict listing approach to its legislation on the control of plastic components,[6] although there is UK legislation controlling vinyl chloride residues in food contact materials, and the migration of vinyl chloride into food (SI 1987 No. 1523). Instead informal controls have been encouraged through the adoption of a Code of Practice.[7] This document, prepared by the British Plastics Federation (BPF) in co-operation with the British Industrial Biological Research Association (BIBRA), was first issued in 1969 and the latest revision appeared in 1988. The Code of Practice contains the following sections:

- Specifications for the composition of sixteen polymers used for food contact applications
- Criteria for the selection of colorants for food contact plastics
- A list of recommended polymer additives with their maximum level of use in the final product.

BIBRA approval for the inclusion of a polymer additive or ingredient in the Code of Practice is based on the available toxicity and migration data for that compound.

The following information is sought:

- Acute toxicity (single dose or multiple doses over 24 hours)
- Short-term toxicity (multiple doses over periods of up to a few months)
- Long-term toxicity (multiple doses over periods of up to the lifespan of the species investigated)
- Effects on reproduction
- Metabolism (including studies on absorption, excretion, tissue distribution, and metabolic route)
- Sensitization potential
- Genotoxicity.

2.3 EC Legislation on Plastic Monomers and Additives

An EC Working Party on Materials and Articles Intended to Come in Contact with Foodstuffs has had the task of reconciling the differing national views on food contact legislation,[6,8,9] for example the adoption of migration limits into foodstuffs *versus* compositional limits for ingredients in the finished product.

The general strategy of the EC is as follows:[9,10,11]

[6] J. D. McGuinness, *Food Add. Contam.*, 1988, **5** (Supplement No. 1), 525.
[7] Plastics for Food Contact Applications. A Code of Practice for Safety In Use. The British Plastics Federation, London with the co-operation of BIBRA. Revised Edition 1986 (including 1988 update).
[8] L. Rossi, *Food Add. Contam.*, 1988, **5** (Supplement No. 1), 543.
[9] L. Rossi, *Food Add. Contam.*, 1988, **5**, 21.
[10] P. A. Tice, *Food Add. Contam.* 1988, **5** (Supplement No.l), 373.
[11] P. A. Tice and J. D. McGuinness, *Food Add. Contam.*, 1987, **4**, 267.

The ratification of an approved list of substances authorized to be used in the manufacture of food contact plastics
An overall migration limit into food for these substances
Specific migration limits for some compounds
A system of checking migration based on standard testing conditions
Compositional limits for some components in the finished material.

References 10 and 11 give more details of the implications of imposing migration limits, and in particular of the UK PIRA Migration Project which was set up to develop analytical methods for monomers, and provide the required migration data.

In order to draw up positive lists of monomers and additives, an EC toxicological advisory committee, the Scientific Committee for Food (SCF), has been evaluating the available toxicity data on the compounds in current use.[8]

In the SCF reports both monomers and additives have been divided into two Annexes: Annex I-substances for which the SCF was able to express an opinion, and Annex II-substances for which there were insufficient data for the SCF to express an opinion. Each Annex was further divided into various lists (Table 1): Lists 0–4 are substances accepted for use, List 5 are banned substances, and Lists 6–9 are compounds for which there are inadequate toxicity data to enable an opinion to be given, or for which the descriptions are inadequate.

Table 1 *Classification of monomers and additives according to the Scientific Committee for Food (SCF)*[a]

Annex I – Substances for which the SCF was able to express an opinion

List 0
Substances which may be used in the production of plastic materials and articles, *e.g.* food ingredients and certain substances known from the intermediate metabolism in man and for which an ADI need not be established for this purpose.

List 1
Substances for which an ADI, or an equivalent classification has been established by the SCF or by JECFA (Joint FAO/WHO Expert Committee on Food Additives).

List 2
Substances for which a TDI or t-TDI has been established by SCF.

List 3
Substances for which an ADI or TDI could not be established, but where the present use could be accepted. Some of these substances are self-limiting because of their organoleptic properties, or are volatile and therefore unlikely to be present in the finished product. For other substances with very low migration, a TDI has not been set but the maximum level to be used in any packaging material is stated. This is because the available toxicological data would give a TDI very much higher than the maximum likely intakes arising from present uses of the monomer or additive.

Table 1 *(cont.)*

List 4 (for additives)
Substances for which an ADI or TDI could not be established, but which could be used if the substance migrating into food or food simulants is not detectable by an agreed sensitive method.

List 4 (for monomers):
List 4A (for monomers)
As list 4 for additives (see above)
List 4B (for monomers)
Substances for which an ADI or TDI could not be established, but which could be used if the levels of monomers residues in materials and articles intended to come into contact with foodstuffs are reduced, as much as possible.

List 5
Substances which should not be used.

Annex II - Insufficient data for the SCF to express an opinion

List 6
Substances for which there exists suspicion about their toxicity and for which data are lacking or are insufficient.

List 6A
Substances suspected to have carcinogenic properties. These should not be detectable in food or in food simulants by an appropriate sensitive method for each substance.
List 6B
Substances suspected to have toxic properties (other than carcinogenic). Restrictions may be indicated.

List 7
Substances for which some toxicological data exist, but for which an ADI or TDI could not be established. The additional specified information should be furnished.

List 8
Substances for which no or only scanty and inadequate data were available.

List 9
Substances and groups of substances which could not be evaluated due to lack of specificity. These groups should be replaced by individual substances actually in use.

ADI = acceptable daily intake; TDI = tolerable daily intake;
t-TDI = temporary tolerable daily intake.
[a] Taken from EEC Document III/3141/89-EN (Rev.3) (CS/PM/500) Brussels 25 July 1990; (*BIBRA Bulletin*, 1990, **29**, 277).

As a result of all this activity a Directive containing lists of permitted monomers was issued in 1990 (90/128/EEC). A draft list of approved additives has also been prepared and should be finalised in the near future.[12] EC Directive 90/128/EEC (23rd February 1990 as corrected on 13th December 1990) divides monomers and other starting substances into

[12] *BIBRA Bulletin*, 1990, **29**, 277; EEC Document III/3141/89-EN (Rev.3) (CS/PM/500) Brussels, 25 July 1990.

two categories: Section A, the so called 'Community List', which contains authorized monomers (155 compounds drawn from SCF lists 0–4) and Section B, the 'Optional National List' (368 compounds drawn from SCF lists 6–9). Entries in Section B may continue to be used pending a decision on their inclusion in Section A and while the toxicity and descriptive data requested by the SCF are being collated, but after January 1st 1993 only substances in Section A will be allowed for the manufacture of plastics as food contact materials.

For monomers and additives presently assigned to Lists 6 and 7, the SCF hopes to see toxicological and migration tests completed according to the following timetable so that acceptable daily intakes can be established:[12]

Hydrolysis, migration, mutagenicity studies: 31st December 1990 (monomers), 31st December 1991 (additives); (hydrolysis studies are intended to show that a compound can be hydrolysed *in vitro* to products of either known toxicity, or to intermediate metabolites in man)
28 day and 90 day feeding studies: 31st December 1991 (monomers), 31st December 1992 (additives)
Reproductive and teratology studies: 31st December 1992 (monomers), 31st December 1993 (additives)
Long-term studies: 31st December 1995 (monomers), 31st December 1996 (additives).

Less toxicity data are required by the SCF if there is analytical evidence that a compound has a low specific migration (EEC Document III/3568/89-Final). These requirements are explained in Table 2.

Table 2 *SCF toxicity requirements where specific migration data are available*[a]

Migration data (p.p.m.)	*Toxicological tests requested*	*Decision*
0–0.050	Three mutagenicity tests	If positive not to be used; if negative R^* = 0.050 p.p.m.
0.050–5 and no suspicion of, for example, carcinogenicity or reproductive toxicity	90 day oral test Three mutagenicity tests Test of biological bioaccumulation	Dependent on toxicological results
5–60 or migration data not presented	Full 'core' set of toxicological tests[b]	Dependent on toxicological results

R^* = restriction recommended by SCF and which can be expressed as specific migration limit or as a mg kg^{-1} body weight or in another manner.

[a] Taken from a Note for guidance of applicants for presentation of a request for assessment of a substance to be used in plastic materials and articles intended to come into contact with foodstuffs. EEC Document III/3568/89 – Final (Brussels 25/7/1990).

[b] Mutagenicity studies; 90 day and long-term toxicity studies; reproductive and teratogenicity studies; absorption, distribution, metabolic, and excretion studies.

2.4 Future Developments

EC legislation on food contact plastics has yet to become law in the UK, though it is expected that the EC monomers directive 90/128/EEC and EC directives on migration testing (82/711/EEC) and recommended food simulants for migration tests (85/572/EEC) will be enacted in UK law shortly (by means of a Statutory Instrument under the 1990 UK Food Safety Act).

With the harmonization of national and EC legislation, the BPF/BIBRA Code of Practice will play a less influential role in advising UK manufacturers and users of plastics. It will continue, however, to recommend ingredients and materials which are not yet covered by EC directives: for example, colorants, processing aids, and the composition of laminates not exclusively composed of plastics.

2.5 Informal UK Controls: Surveillance of Plasticizers in Food

The work of the UK Steering Group on Food Surveillance was mentioned in Chapter 4. Of the thirty 'Food Surveillance Papers' so far published by this group, seven have been concerned with the components of food contact materials. Four of these seven reports have dealt with monomers,[13] and of the last three reports two have summarized analytical data on plasticizer levels in food and also have reviewed the literature on the toxicology of these plasticizers.[14a,14b]

The major plasticizers currently used in food packaging materials are di-(2-ethylhexyl) adipate (DEHA) and polymeric plasticizers (polyesters of dicarboxylic acids and dihydric alcohols, average molecular weight 2000) both of which are used in PVC film, epoxidized soya bean oil (ESBO), acetyl tributyl citrate (ATBC) (used in VDC copolymer film), and di-(2-ethylhexyl) phthalate (DEHP), di-isodecyl phthalate (DIDP), and di-iso-octyl phthalate (DIOP) (used in closure seals for jars).

[13a] Ministry of Agriculture, Fisheries and Food, Report of the Working Party on Vinyl Chloride. Second Report of the Steering Group on Food Surveillance, HMSO, London, 1978.

[13b] Ministry of Agriculture, Fisheries and Food, Report of the Working Party on Acrylonitrile and Methacrylonitrile. Sixth Report of the Steering Group on Food Surveillance, HMSO, London, 1982.

[13c] Ministry of Agriculture, Fisheries and Food, Report of the Working Party on Vinylidene Chloride. Third Report of the Steering Group on Food Surveillance, HMSO, London, 1980.

[13d] Ministry of Agriculture, Fisheries and Food, Report of the Working Party on Styrene. Eleventh Report of the Steering Group on Food Surveillance, HMSO, London, 1983.

[14a] Ministry of Agriculture, Fisheries and Food, Report of the Working Party on Chemical Contaminants from Food Contact Materials: Sub-Group on Plasticizers, Survey of Plasticizers Levels in Food Contact Materials and in Foods. Twenty-first Report of the Steering Group on Food Surveillance, HMSO, London, 1987.

[14b] Ministry of Agriculture, Fisheries and Food, Report of the Working Party of Chemical Contaminants from Food Contact Materials: Sub-Group on Plasticizers, Plasticizers: Continuing Surveillance. Thirtieth Report of the Steering Group on Food Surveillance, HMSO, London, 1990.

The levels of plasticizers which migrate into food wrapped in PVC film or VDC copolymer film are summarized in the most recent Food Surveillance Paper.[14b] The analytical methodology for this work is described in Chapter 6. Foodstuffs were analysed after retail or simulated household use. Maximum daily intakes of each plasticizer were then calculated from the average level of the plasticizer found in each foodstuff, and the average consumption of that food in the UK diet.[14b] These calculations suggest maximum daily intakes of DEHA, ATBC, ESBO, and polymeric plasticizers of 8.2, 1.5, 1.0, and 0.4 mg respectively, though it is recognized that because of assumptions regarding the packaging of a particular foodstuff the true consumption of each plasticizer will be substantially less than these calculations.[14b]

Concern about exposure to plasticizers in the diet gathered momentum when a long-term study carried out in the USA in the early 1980s indicated that DEHP produced liver tumours if administered at high doses in the diet to male and female rats and mice,[15] and that DEHA at high doses produced similar effects in female mice and possibly in male mice.[16]

In their review of the toxicology of DEHA, ATBC, ESBO, and polymeric plasticizers, which accompanied the analytical surveillance of these substances in the diet,[14b] the UK Committee on Toxicity (CoT) noted that there is now a 6000-fold or more margin between the maximum estimated dietary intake of DEHA and the dose which produced carcinogenic effects in mice. The CoT were satisfied with the toxicological profile now available for DEHA (although they did request that a specific genotoxicity test – an intraperitoneal dominant lethal study in rodents – should be carried out), but were critical of gaps in the available toxicity data for the other three plasticizers which they requested should be completed by 1991 or 1992 depending on the test.

Future regulatory control of food contact plastics will increasingly require the packaging industry to supply toxicity data on current and new formulations of plastic ingredients to a defined timetable. In order to secure further development in the range and properties of plastics which can be used to protect food some preliminary assessment of likely toxicity problems would seem prudent.

Computer-aided predictions of toxicological endpoints using structure–activity relationships (SAR)[17] could be a useful tool for the assessment and selection of new polymer additives and monomers. SARs have been successfully used to correlate the short-term hepatic effects of phthalate plasticizers (including those used in food contact materials) and

[15] NTP Technical Report Series 217. Carcinogenesis Bioassay of Di(2-ethylhexyl) phthalate (CAS No. 117-81-7) in F344 Rats and B6C3Fl Mice (Feed Study), NIH Publication No. 82-1773, 1982.

[16] NTP Technical Report Series 212. Carcinogenesis Bioassay of Di(2-ethylhexyl) adipate (CAS No. 103-23-1) in F344 Rats and B6C3Fl Mice (Feed Study), NIH Publication No. 81-1768, 1980.

[17] R. Purchase, J. Phillips, and B. G. Lake, *Food Chem. Toxicol.*, 1990, **28**, 459 and references therein.

their metabolites, with molecular structure. The remainder of this chapter describes the background to this work and the development of SAR for phthalic acid diesters, their metabolites, and some other compounds which produce similar hepatic effects in rodents.

3 SAR and the Components of Food Contact Materials

3.1 SAR and Phthalic Acid Esters

Phthalic acid esters now have a limited use in food contact materials, and the maximum daily intakes of DEHP together with its isomer DIOP from food (bottled beverages) are unlikely to exceed 0.02 mg.[14a] However, the large-scale manufacture of phthalates for use in plastics for other purposes, and the awareness that DEHP is an environmental contaminant, has led to the accumulation of much toxicity data.[18] In particular a considerable effort has been directed towards understanding the mechanisms underlying DEHP's carcinogenic properties in rodent liver, and extrapolating these observations to other species.

DEHP does not appear to be directly genotoxic as it is not mutagenic in the Ames test,[19] nor is there evidence that it or its metabolites bind covalently to hepatic DNA after administration *in vivo*.[20] In short-term feeding studies in rats and mice, DEHP, administered at dietary levels up to 2% w/w, produces liver enlargement, hepatic peroxisome proliferation, and induction of peroxisomal and microsomal fatty acid oxidizing enzymes.[21–23]

Peroxisomes are single membrane-limited cytoplasmic organelles which are common to nearly all eukaryotic cells.[24] Peroxisomes can carry out a diverse range of metabolic functions depending on the cell type, and at least forty enzymes have been found in peroxisomes from different tissues.[25] Irrespective of their origin, all peroxisomes contain at least one hydrogen peroxide (H_2O_2)-generating oxidase, and catalase, an enzyme which converts H_2O_2 into water and oxygen.[22] Amongst other functions, hepatic peroxisomes are capable of oxidizing C_8–C_{22} fatty acids (as their acyl-CoA derivatives) by a β-oxidation pathway.[22] The first enzyme of this pathway, acyl-CoA oxidase, produces H_2O_2, and the cyclic oxidation of a

[18] K. N. Woodward, 'Phthalate Esters: Toxicity and Metabolism', Volumes I and II, CRC Press, Boca Raton, Florida, 1988.
[19] *E.g.* D. Turnbull and J. V. Rodricks, *J. Am. College Toxicol.*, 1985, **4**, 111 and references therein.
[20] *E.g.* A. Von Däniken, W. K. Lutz, R. Jäckh, and C. Schlatter, *Toxicol. Appl. Pharmacol.*, 1984, **73**, 373.
[21] Ref 18. Vol. II, Chapter 1.
[22] J. K. Reddy and N. D. Lalwani, *Crit. Rev. Toxicol.*, 1983, **12**, 1.
[23] E. D. Barber, B. D. Astill, E. J. Moran, B. F. Schneider, T. J. B. Gray, B. G. Lake, and J. G. Evans, *Toxicol. Ind. Health*, 1987, **3**, 7.
[24] P. B. Lazarow and Y. Fujiki, *Ann. Rev. Cell Biol.*, 1985, **1**, 489.
[25] N. E. Tolbert, *Ann. Rev. Biochem.*, 1981, **50**, 133.

single fatty acid molecule will result in the production of several H_2O_2 molecules.[25]

The administration of peroxisome proliferators to rodents produces marked increases in the activities of the enzymes of the hepatic peroxisomal fatty acid β-oxidation cycle.[22,23] Palmitoyl-CoA oxidation in the presence of cyanide ions is used as a specific biochemical marker of this cycle.[26] (Cyanide ions inhibit the competing mitochondrial β-oxidation of fatty acids).[26]

In contrast to the induction of acyl-CoA oxidase and other enzymes of the β-oxidation pathway, and the accompanying production of H_2O_2, H_2O_2-metabolizing enzymes (catalase, cytosolic selenium-dependent glutathione peroxidase) are not markedly induced by peroxisome proliferators.[22]

One explanation for the carcinogenic properties of peroxisome proliferators is that an imbalance in the generation and degradation of H_2O_2 results in increases in the intracellular levels of H_2O_2, leading to the production of reactive oxygen species, oxidative damage to intracellular membranes and/or DNA, and ultimately to the formation of liver tumours.[27,28] However, definitive evidence that this explanation is the primary mechanism responsible for tumour formation is lacking.[29]

In studies on the influence of the alkyl moiety on the peroxisome proliferation properties of phthalates, a series of dialkyl phthalates of varying chain length and branching in the alkyl chain were fed to rats at doses up to 2.5% in the diet for 21 days, and markers of peroxisome proliferation were measured.[23] In the same studies DEHA was also tested, and of the phthalates examined four are used as plasticizers in food contact materials (plastics or regenerated cellulose): DEHP, di-isodecyl phthalate, n-butyl benzyl phthalate, and di-n-butyl phthalate.[14a] The results showed that branched chain esters, namely DEHP, di-isodecyl, and di-isononyl phthalates were more potent peroxisome proliferators than the linear chain phthalates examined (n-butyl benzyl, di-n-butyl, and di-undecyl phthalates). In addition DEHA had a potency comparable with the linear phthalates.[23] Overall there was a ten-fold difference between the weakest and strongest esters in terms of their potency as measured by the induction of cyanide-insensitive palmitoyl-CoA oxidation.[23]

This qualitative relationship between structure and potency was developed in an *in vitro* experiment using five isomeric C_6-phthalic acid monoesters and four isomeric C_8-phthalic acid monoesters.[30] (Phthalic acid diesters are hydrolysed *in vivo* to the monoester and an alcohol; these hydrolysis products and their metabolites are thought to be responsible for

[26] P. B. Lazarow and C. DeDuve, *Proc. Nat. Acad. Sci. USA*, 1976, **73**, 2043.
[27] M. S. Rao and J. K. Reddy, *Carcinogenesis*, 1987, **8**, 631.
[28] J. K. Reddy and M. S. Rao, *Mutation Res.*, 1989, **214**, 63.
[29] B. G. Lake, T. J. B. Gray, A. G. Smith, and J. G. Evans, *Biochem. Soc. Trans.*, 1990, **18**, 94.
[30] B. G. Lake, T. J. B. Gray, D. F. V. Lewis, J. A. Beamand, K. D. Hodder, R. Purchase, and S. D. Gangolli, *Toxicol. Ind. Health*, 1987, **3**, 165.

the hepatic effects of dialkyl phthalates).[21,31]

The linear and branched C_6- and C_8-derivatives included 2-ethylhexyl and cyclohexyl phthalic acid monoesters, whose corresponding diesters are used in food contact materials.[14a]

The monoesters were cultured with rat hepatocytes, and their effects on biochemical markers of peroxisome proliferation, including palmitoyl-CoA oxidation, were determined.[30] All the monoesters produced dose-related increases in enzyme activities, and marked quantitative compound potency differences were also observed. Generally the C_8-isomers were more potent peroxisome proliferators than C_6-isomers, and 2- and 3-ethyl-substituted isomers were more potent than linear and 1-ethyl-substituted analogues. The peroxisomal potency differences found *in vitro* between two branched C_8-isomers (1-ethylhexyl hydrogen phthalate and 2-ethylhexyl hydrogen phthalate) were also observed *in vivo*.[30] Oral administration of these two compounds to rats produced dose-related increases in the activity of palmitoyl-CoA oxidation, with the 2-ethylhexyl isomer being more potent than the 1-ethylhexyl derivative.[30] Similarly the *in vitro* differences found between branched (2-ethylhexyl) and linear (n-octyl) hydrogen phthalates were also reproduced *in vivo* by administration to rats of either the diester or monoester derivatives containing these two alkyl substituents.[32] These *in vivo–in vitro* correlations demonstrate the utility of hepatocytes for detecting differences in peroxisome proliferation potencies which have been observed *in vivo*, and for screening compounds which induce hepatic peroxisomal enzymes.

Quantitative structure–activity relationships (QSAR) were developed from the *in vitro* data for phthalate monoesters; hydrophobic, Taft steric, and electronic parameters were calculated for each compound, and these parameters were related to the potency data (induction of palmitoyl-CoA oxidation) using Hansch-related equations.* Comparatively poor correlations were obtained using hydrophobic and steric descriptors, but by using electronic descriptors for the phthalic acid monoesters a good correlation ($r = 0.968$) with the potency data was achieved.[30] The electronic descriptors were based on MINDO/3 molecular orbital calculations; (the overall total nucleophilic superdelocalizability of the molecules and the electron densities at carbon atoms 6 and 18† were used in the QSAR).[30]

This *in vitro* SAR work was extended to a set of known but apparently structurally diverse peroxisome proliferators.[33,34] The compounds chosen

[31] C. Rhodes, T. Soames, M. D. Stonard, M. G. Simpson, A. J. Vernall, and C. R. Elcombe, *Toxicol. Lett.*, 1984, **21**, 103.

[32] B. G. Lake, T. J. B. Gray, and S. D. Gangolli, *Environ. Health Perspect.*, 1986, **67**, 283.

[33] D. F. V. Lewis, B. G. Lake, T. J. B. Gray, and S. D. Gangolli, *Arch. Toxicol., Supplement 11*, 1987, 39.

[34] B. G. Lake, D. F. V. Lewis, and T. J. B. Gray, *Arch. Toxicol., Supplement 12*, 1988, 217.

*$\log_{10}$(biological activity) $= ax_1 + bx_2 + cx_3 + \ldots k$ where x_1, x_2, x_3, *etc.* are structural, hydrophobic, and electronic descriptors, a, b, c, *etc.* are numerical coefficients obtained by regression analysis, and k is a constant.

†Carbon atom 6 is the aromatic ring carbon atom linked to the alkoxycarbonyl group; carbon atom 18 is the atom linked to oxygen in the alkoxycarbonyl part of the phthalate monoester.

included 2-ethylhexyl hydrogen phthalate but also chemicals not related to those used in food contact materials, such as clofibric acid (a hyperlipidaemic drug) and aryloxy-substituted carboxylic acids. Again electronic descriptors based on molecular orbital calculations for these compounds were successfully correlated with peroxisomal proliferation potency data (induction of palmitoyl-CoA oxidation[33] and catalase[34] in primary rat hepatocyte cultures). In addition, similarities were seen from the molecular graphic plots of the overall shape of some of these compounds,[34] suggesting a three-dimensional structural relationship not revealed from their molecular structures.

This SAR work raises the possibility of predicting rodent hepatic peroxisomal effects from calculable molecular and structural properties for compounds which are being considered as ingredients of food contact materials, and whose toxicology will eventually require scrutiny by the SCF.

3.2 Other SAR Techniques

The Hansch approach to QSAR has therefore been successfully applied to specific biochemical markers of the hepatic effects of a single group of plastic additives. However, Hansch relationships are restricted to structurally coherent compounds, acting by a common biological mechanism. SAR techniques which attempt to predict more complex endpoints (*e.g.* carcinogenicity) for a set of structurally disparate compounds are needed for an effective toxicity screening of new monomers and additives.

Of the various SAR methods which have been applied in toxicology,[17,35] a number are based on the association of sub-structural fragments with biological activity. Recent developments in computer software now allow the generation of structural fragments and the prediction of possible metabolites[36] from a given structure. The merger of this software with SAR methods based on sub-structural analysis could be a useful development, and go some way towards reconciling the demands of regulatory authorities with the capabilities of polymer manufacturers and toxicologists for the evaluation of some of the components of food contact materials.

Acknowledgement

I should like to thank my colleagues at BIBRA, and in particular Dr Brian Lake, for their help with the preparation of this chapter.

[35] L. Turner, F. Choplin, P. Dugard, J. Hermens, R. Jaeckh, M. Marsmann, and D. Roberts, *Toxicol. in Vitro*, 1987, **1**, 143.

[36] F. Darvas, in 'QSAR in Environmental Toxicology-II', ed. K. L. E. Kaiser, D. Reidel Publishing Co., Dordrecht, Holland, 1987, p. 71.

CHAPTER 6

Contaminants from Food Contact Materials: Analytical Aspects

JOHN GILBERT

1 Introduction

Within the European Community, future regulatory control of food contact materials will increasingly depend on the use of positive lists of permitted substances supported by migration testing.[1,2] The use of a particular monomer or additive in a plastic will therefore be controlled, and the amount that leaches or migrates will also be regulated. For the purposes of operating these statutory controls, and to simplify analysis, most testing will be with simulants rather than with real foods. This strategy was first proposed a number of years ago before recent improvements in analytical techniques. The principal aim of using simulants was to simplify analysis to ensure that enforcement could be carried out in most laboratories and could be carried out with commonly available equipment. There has subsequently been much debate over this approach, particularly as to how well simulants actually do 'simulate' real foods.

In order to assess the validity of these food simulants it has proved necessary in many instances to carry out analysis of foods to determine the extent of migration under actual condition of use. Analysis of foods has also proved necessary for surveillance purposes where information is sought on contamination levels in plastic packaged retail samples or foods prepared in the home in contact with plastics. The aim of much of this surveillance is to assess risk by estimating dietary exposure to a particular food contaminant.

[1] L. Rossi, *Food Add. Contam.*, 1988, **5**, 543.
[2] L. Rossi, *Food Add. Contam.*, 1988, **5**, 21.

The ability of the analyst to carry out determinations of low levels of contaminants in real foods has improved significantly in the past few years. This has been largely because of developments in chromatographic techniques, because of improvements in the specificity of detection methods and through novel applications of chemical approaches to sample preparation. It is these new strategies that are reviewed in this chapter, divided into four areas:

(1) The analysis of volatile species;
(2) The analysis of low molecular weight non-volatile species;
(3) The analysis of high molecular weight polydisperse species that can be degraded and can therefore be analysed as low molecular weight entities;
(4) The analysis of high molecular weight polydisperse species that are non-degradable and have therefore to be analysed intact using alternative methods.

2 Headspace Analysis of Volatile Contaminants

2.1 Static Headspace

The principle of what is termed 'static' headspace analysis is very simple and has been described in a number of specialized texts and reviews.[3–5] The sample is sealed in a vial, heated until equilibrium partition is established between the sample and the gaseous headspace, and then a fixed volume of the headspace gas is withdrawn for analysis. The key word is 'equilibration', which is essential for quantification. The gaseous sample is then analysed by gas chromatography in the normal way (by packed or capillary column, in the latter case by split injection). Quantification is by construction of a calibration curve by spiking into uncontaminated material or by standard addition to the sample.

The main advantage of headspace analysis is its simplicity – no sample preparation is required except sealing a representative portion of the foodstuff in the vial. The method can be easily automated and the same procedure for analysis of the monomer in a food can also be applied to the analysis of the volatile species in the food contact material itself.

Limits of detection of 1–15 $\mu g\,kg^{-1}$ have been easily achieved for the headspace analysis of various monomers in foods (see Table 1) with the use of flame ionization, nitrogen specific, or electron capture detection as appropriate. In a number of instances there is advantage in using a mass

[3] H. Hackenberg and A. P. Schmidt, 'Gas Chromatographic Headspace Analysis', Heyden, London, New York and Rheine, 1977.
[4] B. Kolb, 'Applied Headspace Gas Chromatography' Heyden, London, New York, Philadelphia, and Rheine, 1980.
[5] B. Kolb, 'Analysis of Food Contaminants', ed. J. Gilbert, Elsevier Applied Science, London, 1984, p.117.

spectrometer as the GC detector, for example for styrene,[6] butadiene,[7] and benzene[8] analysis.

Table 1 *Static Headspace analysis of volatile contaminants in foods*

Contaminant	*Detection method*	*Limit* ($\mu g\ kg^{-1}$)	*Examples*	*Ref.*
Vinyl chloride	FID	1	Cooking oil, orange drink	*a*
Acrylonitrile	AFID	5	Soft margarine	*b*
Butadiene	GC–MS (SIM)	1	Soft margarine	*c*
Styrene	GC–MS (SIM)	1–15	Cream, yoghurt, deserts	*d*
Vinylidene chloride	ECD	5	Biscuits, potato crisps	*e*
Benzene	GC–MS (SIM)	1	Cooked meals	*f*

FID = flame ionization detector; AFID = alkali flame ionization detector; SIM = selected ion monitoring; ECD = electron capture detector.
[a] J. Gilbert and M. J. Shepherd, *J. Assoc. Publ. Anal.*, 1981, **19**, 39.
[b] J. Gilbert and J. R. Startin, *Food Chem.*, 1982, **9**, 243.
[c] J. R. Startin and J. Gilbert, *J. Chromatogr.*, 1984, **294**, 427.
[d] J. Gilbert and J. R. Startin, *J. Sci. Food Agric.*, 1983, **34**, 647.
[e] J. Gilbert, M. J. Shepherd, J. R. Startin, and D. J. McWeeny, *J. Chromatogr.*, 1980, **197**, 71.
[f] S. M. Jickells, C. Crews, L. Castle, and J. Gilbert, *Food Add. Contam.*, 1990, **7**, 197.

The use of the mass spectrometer despite being rather sophisticated as a detection method does offer some particular attractions:

(1) The achievable sensitivity is often better than can be attained with other GC detectors – this means that headspace analysis can be utilized for low volatility compounds with unfavourable partition, for example styrene, where on equilibration the majority of the analyte still remains in solution.
(2) As there is no clean-up prior to headspace analysis, normally reliance is placed entirely on chromatography to ensure separation of the analyte from potential interfering species. Mass spectrometric detection is, however, more specific: the monitoring of a number of chosen ions allows for immediate confirmation and this high confidence in correct assignment of identity enables method development to be carried out rapidly.

Static headspace analysis has been successfully applied to a wide range of food matrices varying from the analysis of, for example, vinyl chloride in foods such as cooking oil stored in PVC bottles[9] to the analysis of benzene in composite foods such as complete microwave meals heated in contact with plastics cookware containing trace levels of benzene contamination.[8]

[6] J. Gilbert and J. R. Startin, *J. Chromatogr.*, 1981, **205**, 434.
[7] J. R. Startin and J. Gilbert, *J. Chromatogr.*, 1984, **294**, 427.
[8] S. M. Jickells, C. Crews, L. Castle, and J. Gilbert, *Food Add. Contam.*, 1990, **7**, 197.
[9] J. Gilbert and M. J. Shepherd, *J. Assoc. Publ. Anal.*, 1981, **19**, 39.

2.2 Dynamic Headspace

In some situations the identity of the volatiles which could be released from the packaging may not be known. In order therefore to characterize these compounds fully (say by mass spectrometry), larger sample sizes are required than can be obtained by the static headspace approach. In these instances 'dynamic headspace' is frequently employed where the headspace gas is exhaustively swept or purged into a trap prior to GC analysis. This can be carried out either employing commercially available equipment or using relatively simple 'home-made'devices.[10,11] This approach has proved useful recently in characterizing volatiles released from microwave susceptor materials. These composites consist of a paperboard substrate, adhesive, aluminium, and PET food contact layer and are used for browning applications in a microwave oven.[12] At the high temperatures produced there are the possibilities not only of volatiles being released from any one of the materials in the composite, but also of charring of the paperboard and crazing of the PET layer. Studies of these materials using dynamic headspace approaches have shown complex chromatogams such as that illustrated in Figure 1 for which there is interest in identification of the compounds prior to assessing whether there is a need for subsequent food analysis.

3 Analysis of Low Molecular Weight Non-volatile Species

Most discrete individual chemical species of low molecular weight that might be present in foods as a result of migration can be determined by procedures involving extraction, sequential clean-up stages usually employing open column chromatography followed by GC or HPLC end-determinations. An initial practical limitation may be the resource requirement for method development, which can prove to be extensive, and a limitation to routine application can be the frequently time-consumimg nature of the clean-up. However, this can be sometimes circumvented by utilizing the high specificity of detection methods such as mass spectrometry and thereby reducing the necessary clean-up. A number of examples from the literature illustrate the analysis in foods of low molecular weight non-volatile species such as antioxidants,[13] stabilizers,[14] and oligomers.[15]

[10] D. L. Heikes, *J. Assoc. Off. Anal. Chem.*, 1987, **70**, 215.
[11] J. Drozd and J. Novak, *J. Chromatogr.*, 1978, **157**, 141.
[12] P. Harrison, *Packaging Technol. Sci.*, 1989, **2**, 5.
[13] R. Goydan, A. D. Schwope, R. C. Reid, and G. Cramer, *Food Add. Contam.*, 1990, **7**, 323.
[14] A. D. Schwope, D. E. Little, D. J. Ehntholt, K. R. Sidman, R. H. Whelan, and P. S. Schwartz, *Deut. Lebensm.-Rund.*, 1986, **82**, 277.
[15] T. H. Begley and H. C. Hollifield, *J. Agric. Food Chem.*, 1990, **38**, 145.

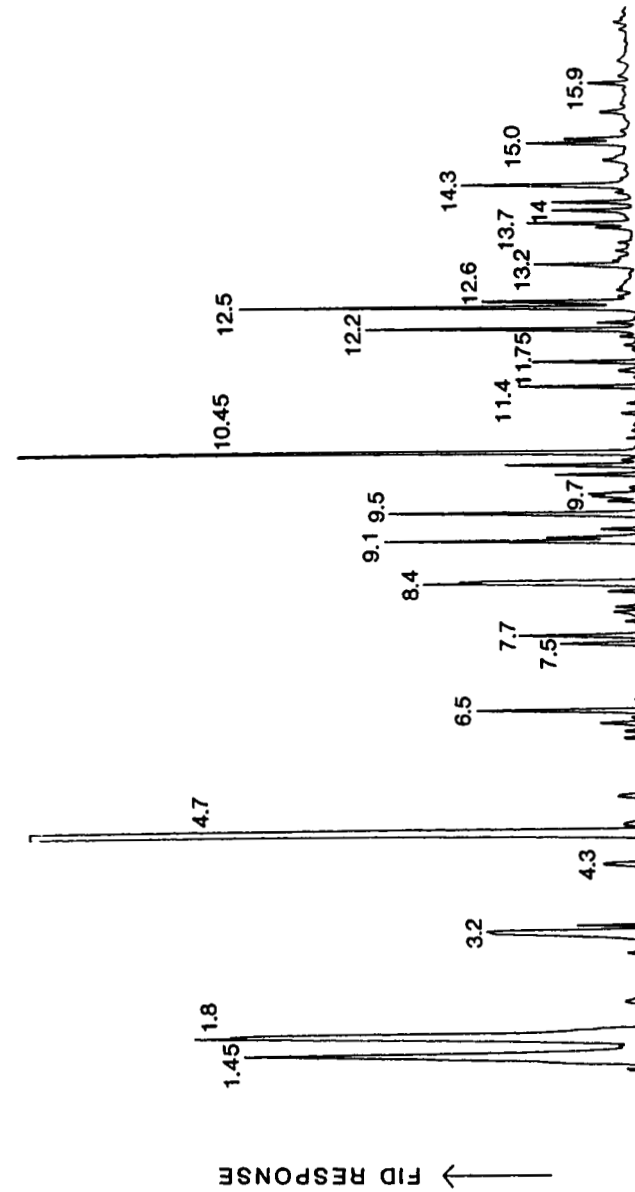


Figure 1 *Capillary GC analysis of volatiles released when susceptor material has been heated for* 4 min *at* 190 °C

3.1 Plasticizer Analysis

Plasticizers are used to provide flexibility to films and to coatings and a variety of types are used for different food contact applications. For PVC film the principal plasticizer has in the past been di-(2-ethylhexyl) adipate (DEHA)[16] although this has now being partially replaced in films by so-called polymeric plasticizers.[17] This type of 'cling-film' is widely used in the home for wrapping and covering foods, and in supermarkets for covering fresh and cooked meat, fruit, and some vegetables. PVC/PVDC copolymer is a flexible film sold specifically for covering foods during cooking in a microwave oven and this is plasticized with acetyltributyl citrate. Phthalates (mainly dibutyl, butylbenzyl, and dicyclohexyl) are used to provide flexibility to the nitrocellulose coating of regenerated cellulose film which finds very wide application for the packaging of confectionery for cooked meat pies and pasties, and for cakes.[18]

The analysis of plasticized film is carried out by a simple solvent extraction of the plastic with chloroform containing one or more internal standards and then a GC determination. Identification of the plasticizers can usually be made on the basis of retention times compared with standards.

The analytical approach to determining plasticizers in foods is shown in schematic form in Figure 2. The plasticizers are all lipid soluble, so the method essentially involves fat extraction and then separation of the higher

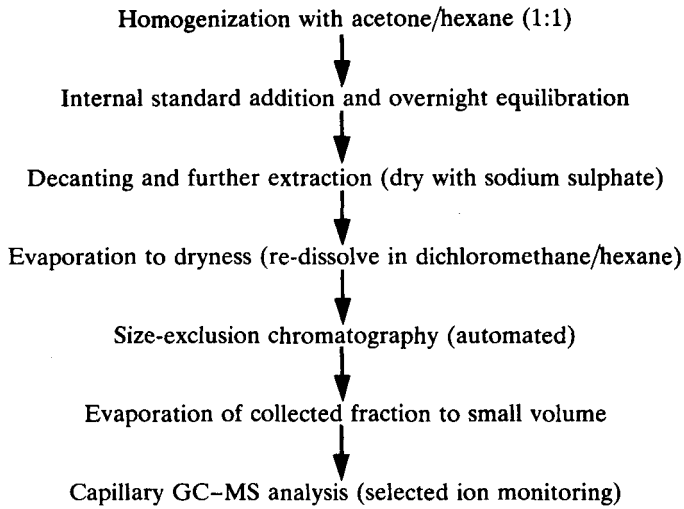


Figure 2 *Schematic illustrating the analysis of plasticizers in foods*

[16] J. R. Startin, M. Sharman, M. D. Rose, I. Parker, A. J. Mercer, L. Castle, and J. Gilbert, *Food Add. Contam.*, 1987, **4**, 385.
[17] L. Castle, A. J. Mercer, and J. Gilbert, *Food Add. Contam.*, 1988, **5**, 277.
[18] L. Castle, A. J. Mercer, J. R. Startin, and J. Gilbert, *Food Add. Contam.*, 1988, **5**, 9.

molecular weight lipids from the lower molecular weight plasticizers by size exclusion chromatography (SEC). The SEC stage of the analysis has the advantage of being amenable to automation. Analysis of the cleaned-up extract is by capillary GC. Although GC analysis using a flame ionization detector (FID) can be effective for some foods, for others, for example confectionery products and some complete cooked meals, interference peaks will be present, obscuring the region of the chromatogram where the plasticizers are expected to elute. In these instances, it is advisable to employ mass spectrometric selected ion monitoring. This has the added advantage that stable isotope-labelled internal standards can then be employed, and the assay becomes one of isotope dilution.[19,20] Although the use of the mass spectrometer does require a high level of sophistication of instrumentation, for large numbers of survey samples, data can be generated quickly, with a high precision and with a high confidence in correct identification of the plasticizers being monitored.

3.2 Analysis of Glycol Softeners

Glycol softeners are used in regenerated cellulose film (RCF) to provide flexibility and are typically used at levels up to 27% w/w in the film. Prior to 1986 monoethylene glycol (MEG) and diethylene glycol (DEG) were widely employed, but have now been replaced by alternative softeners. The softeners now most frequently used are combinations of two or more of the following: propylene glycol, triethylene glycol, poly(ethylene glycol), glycerol, and urea. Although MEG and DEG are still permitted as RCF softeners, recent EC Regulations now restrict the total level of MEG plus DEG in the food as a result of migration not to exceed a limit of $50\ mg\ kg^{-1}$.

Glycol softeners in RCF can be extracted into acetone or acetonitrile, with the inclusion of butane-1,4-diol as an internal standard, derivatized with silylating reagent, and then analysed by GC. All the glycols can be quantified by this analysis except for poly(ethylene glycol) where only its lower molecular weight components can be detected, although these could be used as a basis for the determination.

The analysis of glycols in foods is particularly difficult because of problems of efficient extraction from the food matrix, the similarity in behaviour of the glycols to other polar molecules present in foods, and the lack of a suitable chromophore or other 'handle' for detection. An effective method has been developed[21] which although rather lengthy can, when applied with care, give good results for the determination of glycols in chocolate and other confectionery products. The method which is shown in schematic form in Figure 3, involves extraction into hot water, defatting

[19] J. R. Startin, I. Parker, M. Sharman, and J. Gilbert, *J. Chromatogr.*, 1987, **387**, 509.
[20] L. Castle, J. Gilbert, S. M. Jickells, and J. W. Gramshaw, *J. Chromatogr.*, 1988, **437**, 281.
[21] L. Castle, H. R. Cloke, J. R. Startin, and J. Gilbert, *J. Assoc. Offic. Anal. Chem.*, 1988, **71**, 499.

with hexane, precipitation of the sugars, TMS derivatization, and finally analysis by capillary GC with an FID. Most problems in this analysis are caused by contamination occurring in solvents, reagents, or from glassware and particular care is necessary in this aspect of sample preparation. Analytical quality assurance checks are therefore essential in the form of frequent analyses of blank samples.

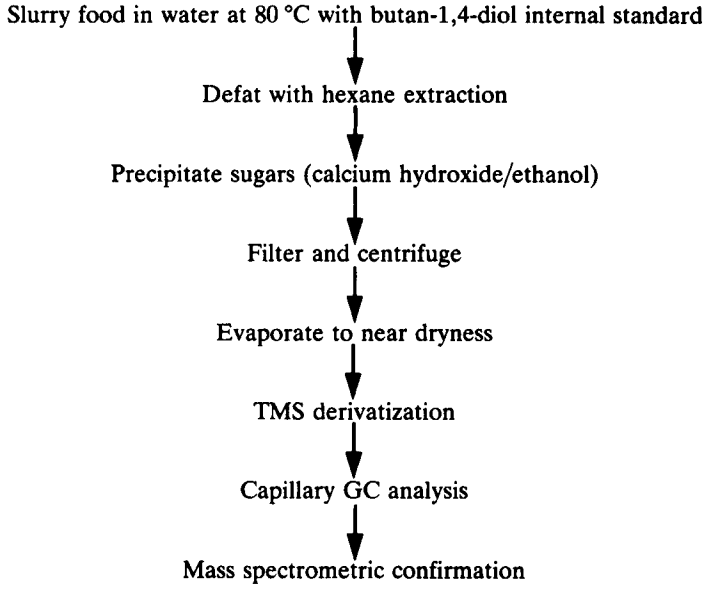


Figure 3 *Schematic illustrating the analysis of glycol softeners in foods*

3.3 Monomer Analysis

A number of monomers are not sufficiently volatile for headspace analysis, and in some instances where they contain a suitable chromophore HPLC is the appropriate technique to employ. 4-Chlorophenylsulphone is one of the starting substances for the manufacture of polyethersulphone. This monomer can be determined in foods by using a multidimensional HPLC system, where size exclusion and reverse phase chromatography are coupled on-line.[22] In the example shown in Figure 4 the 4-chlorophenylsulphone monomer can be detected at 0.1 $mg\,kg^{-1}$ in olive oil using HPLC with UV detection, by direct analysis of the oil without a need for extraction or any preliminary clean-up.

[22] R. A. Williams, R. Macrae, and M. J. Shepherd, *J. Chromatogr.*, 1989, **477**, 315.

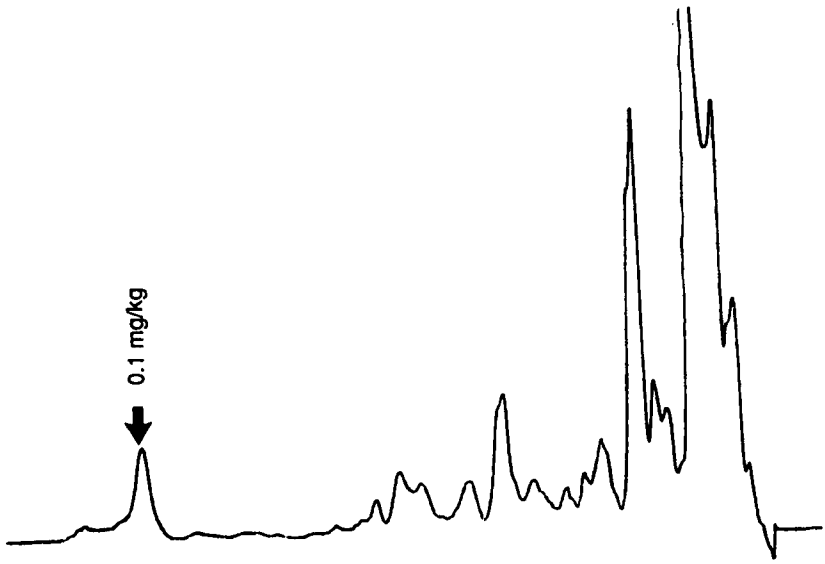


Figure 4 *HPLC analysis of 4-chlorophenyl sulphone monomer spiked at* 0.1 mg kg^{-1} *in olive oil. Automated system employed involving coupled high performance size exclusion chromatography and reverse phase HPLC with UV detection*

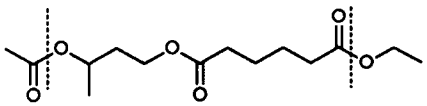
4 Analysis of Polydisperse Degradable High Molecular Weight Species

There are many constituents of plastics that are not single species but consist of a distribution of components covering a wide range of molecular weights. Where these polydisperse compounds contain repeat units and are amenable to some form of degradation then the possibility exists of converting to one or more of the constituent parts and then determining these using similar methods to those outlined above. Three examples of plastics additives illustrate this approach.

4.1 Polymeric Plasticizer Analysis

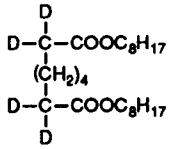
Polymeric plasticizers may be used as partial or complete replacements for conventional plasticizers such as DEHA in PVC cling-film[17]. A typical example of a polymeric plasticizer is a polyester of butane-1,3-diol and adipic acid as shown (1). This plasticizer is in fact a complex mixture of oligomers and has a number average molecular weight of around 1950 – approximately five times greater in relative mass than DEHA.

To determine the migration of polymeric plasticizer, deuterated DEHA



Reoplex 346:
polyester of butane-1,3-diol and adipic acid

(1)



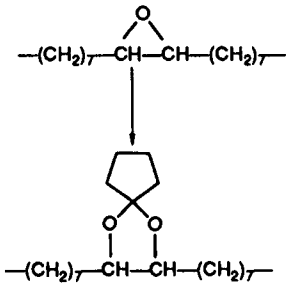
(2)

(2) is employed as an internal standard. The approach[23] is based on initial transmethylation of the polymeric plasticizer to form dimethyl adipate (DMA). At the same time the deuterated-DEHA internal standard is also transmethylated to deuterated-DMA, and after automated SEC clean-up, the DMA and [$^2H_4$]-DMA are simultaneously measured by selected ion monitoring GC–MS. The limit of detection for the polymeric plasticizer in foods was conservatively set at the lowest level of interest which was 0.1 mg kg^{-1} and the assay was shown to have a precision of about 4%. The method is such that for the analysis of foods containing both DEHA and polymeric plasticizer the assay gives a total level for the two components. If the DEHA is then determined separately the level of polymeric plasticizer in the food can be determined by difference from the total. The method has a high specificity and has been used to analyse a diverse range of foods such as sandwiches, meat, biscuits, cake, and complete microwave cooked meals that have been in contact with plasticized film.

4.2 Epoxidized Soya Bean Oil Analysis

Epoxidized soya bean oil (ESBO) is used as a plasticizer and secondary heat stabilizer in flexible PVC applications at levels of between 3 and 7%, as well as in other plastics where it might, for example, function as a lubricant. The approximate component fatty acid composition of ESBO is 50% diepoxidized, 26% monoepoxidized, and 11% triepoxidized components. An analytical approach to ESBO analysis is possible based on the similar principle of degradation of the triglyceride to its constituent fatty acid components.[24] The approach involving transmethylation under basic conditions converts the triglyceride into its component fatty acid methyl esters. These fatty acid esters do not receive further clean-up from the co-extracted food lipids but are instead derivatized to form 1,3-dioxolanes (3) which are then amenable to direct GC–MS analysis. Quantification is based on an estimation of the diepoxidized fatty acid esters. This assay has a sensitivity and precision similiar to the procedure for monomeric plasticizers and has been applied to a wide range of food types[25] subject to satisfactory blank values being obtained for food samples not having had prior contact with ESBO-plasticized material.

[23] L. Castle, A. J. Mercer, and J. Gilbert, *J. Assoc. Offic. Anal. Chem.*, 1988, **71**, 394.
[24] L. Castle, M. Sharman, and J. Gilbert, *J. Assoc. Offic. Anal. Chem.*, 1988, **71**, 1183.
[25] L. Castle, A. Mayo, and J. Gilbert, *Food Add. Contam.*, 1990, **7**, 29.

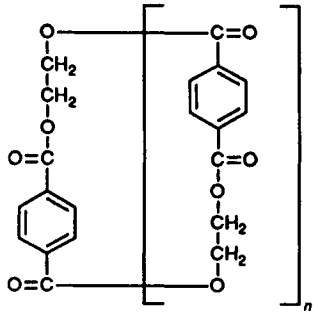


(3)

4.3 Analysis of Poly(ethylene terephthalate) Oligomers

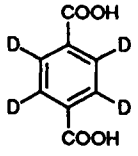
Another application of the degradative approach to a single species is the analysis of poly(ethylene terephthalate) (PET) oligomers in foods. PET is being increasingly used both for retail applications such as beverage bottles and for trays and cookware for microwave oven applications. Migration from PET is known to be very low and what little material does migrate probably consists of oligomers of PET, of which the cyclic structures (4) are the most prominent.

The method to measure the total PET oligomer plus terephthalic acid monomer concentrations in foods uses tetradeuterated terephthalic acid as internal standard (5). As with the two schemes outlined above for analysing higher molecular weight analytes, this approach again relies on degradation to a common species.[26] In this instance dimethyl terephthalate is formed both from the complex mixture of oligomers as well as from the deuterated internal standard. Clean-up and GC–MS determination is similar to that described for the other applications. The method has a limit of detection of 0.1 mg kg^{-1}, has a relative standard deviation of 5%, and is applicable to a range of foods from oils and beverages to complete composite meals.



Major cyclic oligomers n = 1-3

(4)



(5)

[26] L. Castle, A. Mayo, C. Crews, and J. Gilbert, *J. Food Protect.*, 1989, **52**, 337.

5 Analysis of Polydisperse Non-degradable High Molecular Weight Species

The final group of compounds which are the most troublesome analytically are those polydisperse species, frequently of relatively high molecular weight, which cannot be broken down into useful smaller portions for analysis. They therefore have to be analysed as a group either by GC, first separating the components and then summing them together to assess the total amount, or by HPLC without separation; both approaches can have disadvantages of poor sensitivity, detection lacking specificity, and poor precision. A recent example that illustrates these problems is the analysis of mineral hydrocarbons in foods.

Mineral hydrocarbons are hydrocarbon fractions derived from petroleum and include mineral oils, liquid paraffins, petroleum jelly, and microcrystalline waxes. They thus encompass a wide molecular weight range and vary from relatively simple mixtures of hydrocarbons that can be resolved chromatographically to complex mixtures that are unresolvable and elute as a broad single component spread over a wide portion of the chromatogram. Food contact applications of mineral hydrocarbons include coatings on cheeses, lubricants for the casings of skinless sausages, and for incorporation in plastics formulations as flow promoters. In this latter application amounts in the range *ca.* 0.5–6.0% of mineral hydrocarbons may be employed, for example in polystyrene cups, tubs, and containers that have a wide range of intended applications in contact with aqueous and fatty foods for normal as well as high temperature applications.

The simplest example of analysis of mineral hydrocarbons has been the study of the migration into cheese from the wax coating. Cheese samples have been extracted into carbon tetrachloride and the hydrocarbon contaminants separated from polar material by a single-stage clean-up using a silica cartridge. The extract was then analysed directly by capillary GC. The lack of complexity of these particular hydrocarbons is illustrated in Figure 5. This means that it is possible to separate individual mineral hydrocarbon components by GC, ensure that there are no interferences from the cheese, and then measure the total mineral hydrocarbon migration by summing the peak areas. In this example C_{16} hydrocarbon was added as an internal standard. The analysis of a core sample of the cheese remote from the wax provided an effective 'blank' sample, and when the chromatogram from this sample was compared with that of a cheese sample obtained from the immediate contact layer with the wax, it was clearly demonstrated that a migration phenomenon was involved.

Most other mineral hydrocarbons that are used in food contact applications have a considerable complexity such that it is not possible to separate the mixtures into individual components. The hydrocarbons thus elute from a GC column as a broad peak which is difficult to quantify, although by the addition of a mixture of internal standards, covering the molecular weight range of interest, it is possible to make some quantitative assess-

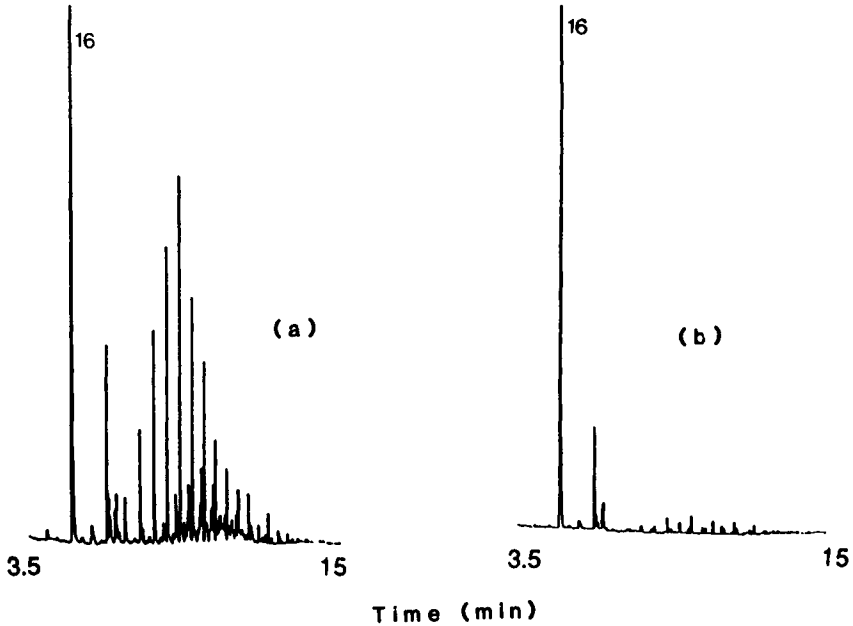


Figure 5 *Capillary GC analysis of mineral hydrocarbon migration from wax coating into cheese. Peak numbered 16 is* C_{16} *internal standard.* (a) *Analysis of a sample of cheese cut* 1–2 mm *from the wax coating;* (b) *Analysis of a blank sample of cheese obtained from the core of the sample (no contact with wax)*

ment. Figure 6 illustrates the analysis of mineral hydrocarbons in a chocolate drink which has been in contact with a polystyrene vending machine cup containing mineral hydrocarbons. It can be clearly seen from the chromatogram where the drink has been spiked with $0.5\,mg\,kg^{-1}$ of mineral hydrocarbons that with such a broadly eluting peak there can be no guarantee that food components are not being co-eluted under the peak. Determinations can therefore only be carried out on migration into pre-selected foods which are known to be free of background interferences. The alternative approach of HPLC, although producing a single rather sharper eluting component, lacks sensitivity because of reliance on refractive index detection and still provides no assurance of adequate specificity. Work with these components cannot be carried out on retail samples for surveillance and data can only be generated by setting-up migration experiments.

The best approach to providing reliable migration data into a variety of foods involves incorporating radiolabelled hydrocarbons or fortifying with a mixture of discrete hydrocarbons into the plastics formulation during the fabrication of the containers. This does have the disadvantage that the containers are not commercial products and one is limited to a single

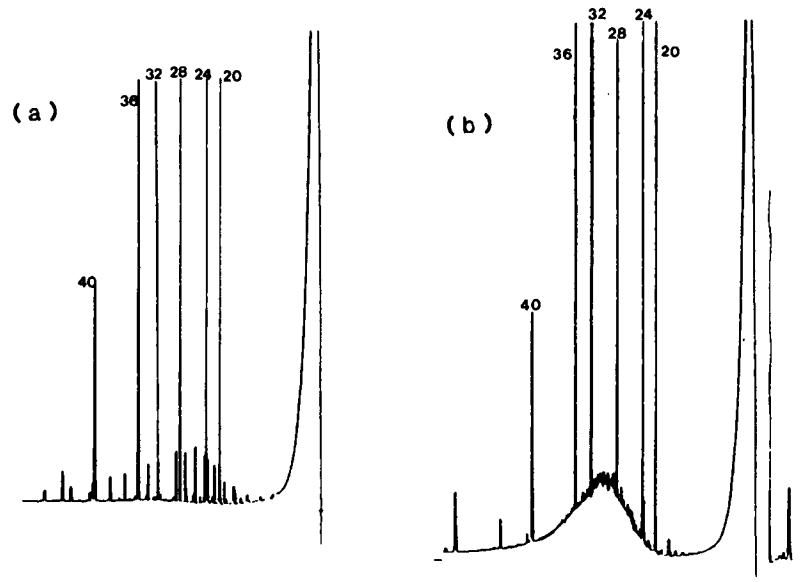


Figure 6 *Capillary GC analysis of mineral hydrocarbon migration from a polystyrene vending machine cup into a hot chocolate drink. Peaks numbered 20–40 are internal standards* C_{20} *to* C_{40}. (a) *Chocolate drink containing* $< 0.5\ mg\,kg^{-1}$ *total mineral hydrocarbon content;* (b) *Chocolate drink spiked with a total level of* $0.5\ mg\,kg^{-1}$ *of mineral hydrocarbons*

formulation. However, a rapid assessment can be made of migration into a diversity of food types and when using radiolabelled compounds there is no pre-requisite that the food should be free of any analytical interferences.

6 Conclusions

This chapter aims to demonstrate that most contaminants that migrate from food contact materials can be analysed at low levels in real foods. The volatile individual species are the easiest to determine because of the possibility of employing the headspace approach. There are, however, strategies emerging for the analysis of the more intractable mixtures that may contaminate foods, although achieving adequate sensitivity and specificity can be a problem. These developments in analytical techniques now enable a far better assessment of migration to be made under realistic conditions of use of food contact materials than has previously been possible.

CHAPTER 7

Use and Regulatory Control of Veterinary Drugs in Food Production

KEVIN N. WOODWARD

1 Introduction

Veterinary medicines, like their human counterparts, are intended to improve or maintain the health of animal species whether these are kept for food production or otherwise. Thus they cover a variety of therapeutic and prophylactic purposes and are administered to food-producing animals, to household pets, and to exotic species. The diseases for which medicines are used range from bacterial infections requiring antibiotic or antimicrobial therapy to external parasitic infestations necessitating topical or immersive treatments with organophosphorus compounds. Many veterinary drugs are administered in an identical manner to human drugs, for example by tablet, injection, or topical application. Others, however, are given in ways which would be unacceptable for human use such as the sheep dips, slow-release anthelmintic formulations, or insecticidal collars for household pets. All medicines used in the UK, whether for veterinary or human use have several things in common. They are all designed to be biologically active, many are potentially toxic, and all are regulated under the Medicines Act 1968 and associated European legislation.

The Medicines Act presents a comprehensive system of licensing for human and veterinary medicinal products, and to qualify for a product licence a medicine must satisfy three criteria: safety, quality, and efficacy. Taking these in reverse order, efficacy is the ability of the drug to accomplish the task that the licence applicant has set for it. Does it work as an antibiotic, as a growth promoter, as an anthelmintic? Quality in this context refers to pharmaceutical quality. The drug must be free from unacceptable contaminants, have the shelf-life and isomeric content claimed for it, and be manufactured to appropriate standards. Safety refers

to the inherent ability of a drug product to cause harm, or more specifically not to cause harm to biological systems which become exposed to it. Of course both quality and efficacy have safety implications. An impure drug may contain toxic contaminants or a poorly efficacious medicine may result in a lethal outcome to the disease. Safety on the other hand considers the inherent properties of the medicine. It covers safety to the patient whether human or animal, safety to the operator including veterinarians, farmers, and members of the public, and safety to the environment. In the case of medicines intended for use in food-producing animals, safety to consumers of products containing drug residues is of paramount importance. It is largely this latter aspect which will form the substance of this chapter.

2 Medicines Legislation

Apart from some extremely old statutes governing the quality of medicines, there was no effective control of medicines in the UK prior to 1968.[1] However, following the disastrous sequence of events brought about by the use of the drug thalidomide in the 1960s, the Committee on Safety of Drugs under the Chairmanship of Sir Derek Dunlop was formed, and later drug scrutiny was put on a statutory basis by the passing in Parliament of the Medicines Act 1968.

Similarly, prior to 1968, veterinary drugs had been controlled in the UK by a voluntary scheme aimed at ensuring appropriate safety labelling with the intention of protecting humans, livestock, domestic animals, and wildlife against any potential adverse effects. The Ministry of Agriculture, Fisheries and Food which oversaw the scheme was advised on these aspects by the Advisory Committee on Pesticides and Other Toxic Chemicals and by the Veterinary Products Committee.[1] After 1968, veterinary products were regulated under the Medicines Act.

The Medicines Act provides for a Licensing Authority which has the power to grant – or otherwise – a product licence. The Licensing Authority is defined in the Act as the Health and Agriculture Ministers acting in a joint manner. It is advised on safety, quality, and efficacy matters by expert independent committees established under Section 4 of the Act. For human medicines the Committee on Safety of Medicines provides the advice whilst for veterinary drugs the Veterinary Products Committee (VPC) serves this purpose.

The function of the Licensing Authority is devolved to the Medicines Control Agency (MCA) of the Department of Health for human medicines and to the Veterinary Medicines Directorate (VMD), an Executive Agency and a part of the Food Safety Directorate of the Ministry of Agriculture,

[1] M. F. Cuthbert, J. P. Griffin, and W. H. W. Inman, in 'Controlling the Use of Therapeutic Drugs. An International Comparison', ed. W. M. Wardell, American Institute for Public Policy Research, Washington DC, 1978, p. 99.

Fisheries and Food, for veterinary products. Staff in the MCA and VMD provide scientific and administrative secretariats which service the independent expert committees, carry out assessments of safety, quality, and efficacy data, take part in national and international discussions of drug regulation, and issue the product licences and other forms of market authorization provided for under the legislation.

Applications for product licences for veterinary medicines are sent to the VMD which is based on the site of the Central Veterinary Laboratory in Weybridge, Surrey. Specialized assessments of the safety, quality, and efficacy data are carried out by toxicologists, pharmacists, and veterinarians on the staff of the Directorate and reports are prepared which are initially considered by a committee known as the Scientific Secretariat. This is composed of members of the VMD staff and experts drawn from other parts of the Ministry of Agriculture, Fisheries and Food including the Central Veterinary Laboratory and Food Science Division as well as from other government agencies including the Department of Health and the Health and Safety Executive. The Committee has no statutory basis but it serves to give an initial and exceptionally thorough analysis of the data supplied in support of applications, identifying problem areas and shortcomings. The reports, modified where necessary in the light of the Scientific Secretariat's deliberations, are then forwarded for independent expert advice to the VPC.

The simplest outcome to a VPC consideration is to recommend that a product licence be granted. However, factors often prevail which prevent this conclusion. There may be inadequate or insufficient data, more information may be required, or the Committee may decide that the product is not safe, efficacious, or of sufficient quality – or of course a combination of these. Under these circumstances the Committee writes to the applicant under Section 21 of the Medicines Act informing the company concerned that it may not be able to advise the Licensing Authority to grant a licence and listing the reasons for this decision. The applicant is then offered the opportunity to make written representations or to appear before the Committee in order to resolve the problems. This is commonly referred to as the appeals procedure, and if unsuccessful the applicant is given a final opportunity to appeal before the Medicines Commission, a broadly based committee appointed under Section 2 of the Act. The Medicines Commission may uphold or overturn the decisions of the VPC. When the 'appeals' procedure is exhausted the applicant may pursue his case by judicial review.

The Licensing Authority also has powers to deal with existing product licences when adverse effects become apparent or if new data indicate that the product can no longer be regarded as safe, efficacious, or of sufficient quality. For example, a newly published carcinogenicity study may indicate that an active ingredient or excipient has the potential to induce cancer in laboratory animals. In such cases, the Licensing Authority may revoke the product licence, a process which takes some time and during which period

the product is allowed to remain on the market. However, if it is believed that the effect is life-threatening, then the licence may be suspended with immediate effect whilst revocation proceedings are simultaneously put into effect. For more minor effects, the Licensing Authority may seek to vary the licence compulsorily so as to include additional warnings on the data sheet or to alter the type of packaging. Licence revocation is subject to the appeals procedures described earlier.

An important part of the licensing system is the Adverse Reaction Scheme. This is similar to the 'yellow card' scheme for human drugs and it allows suspected adverse reactions in animals, or indeed in humans if they become contaminated, to be reported to the Licensing Authority. Consequently, alterations to the data sheet, to the product label or to the licensing conditions can then be made to reduce the incidence of, or avert further adverse reactions.

It is important to note that product licences are not the only market authorizations granted for veterinary drugs under the Medicines Act. The Animal Test Certificate (ATC) is the equivalent of the human Clinical Trials Certificate. It is granted largely for the purpose of establishing the efficacy of a proposed new medicine. As the toxicological and residue profiles are usually poorly established when companies apply for ATCs, carcases of treated food-producing animals are often destroyed to prevent them from entering the human food-chain. Companies may also apply for variations to existing product licences or to ATCs to extend these to other indications or to other species. Again, sufficient data must be supplied to support such applications. A current exercise requiring a considerable amount of input by the VMD Secretariat and the VPC arises from the review of medicines. When the Medicines Act 1968 took effect in 1971, products already on the market in the UK were eligible for Product Licences of Right.[2] These were often granted with only a minimum of data. Although they carry the same status as standard product licences, both the Medicines Act and European legislation required that these products should be reviewed. They are essentially being reviewed as if each was a new licence application and applicants must submit safety, quality, and efficacy data to satisfy the Licensing Authority that the products meet modern standards of assessment. As with new applications, the Licensing Authority is advised on review products by the VPC.

3 European Legislation

In the UK, what might be termed conventional medicines, *i.e.* those given orally, by injection, or topically applied for standard therapeutic reasons *and* those given via the medication of feed, are treated in an identical manner using regulations made under the Medicines Act. This is not the

[2] G Jones, 'The Regulation of Medicines in the UK', in 'Pharmaceutical Medicine', ed. D. M. Burley and T. W. Binns, Edward Arnold, London, 1985, p. 149.

case in European Legislation. Conventional medicines are dealt with under the veterinary medicines Directives whereas medicated feeding stuffs are dealt with under a separate Directive. Indeed, each is handled by a separate Directorate General within the European Commission in Brussels. The European legislation governing veterinary medicines will not be discussed at length in this chapter as it as been described in some detail elsewhere.[3] However, the fundamental aspects will be briefly explained in the paragraphs which follow.

The two major Directives which pertain to conventional veterinary medicines in the European Community are Council Directives 81/851/EEC and 81/852/EEC.[4,5] The main objective of the former was to establish the Committee for Veterinary Medicinal Products (CVMP) and of the latter to lay down the regulatory requirements for analytical, toxicological, and pharmacological test guidelines. These have been subsumed into UK law using Medicines Act legislation. The two Directives serve to ensure that regulatory requirements for veterinary medicinal products are the same throughout the Community, whilst the purpose of the CVMP is to determine whether or not a medicinal product complies with the requirements of the Directives. A manufacturer wishing to obtain market authorization in Member States, having already done so in a single State, may follow the so-called CVMP procedure whereby his application is forwarded to that Committee's Secretariat in Brussels and to five other member states for consideration. The opinion of the CVMP will be sought if any of the States raises an objection on safety, quality, or efficacy grounds. If a favourable opinion is given, Member States must then individually decide on granting marketing authorization, and the CVMP is informed accordingly. It is compulsory for all high technology products, namely those derived from recombinant DNA technology or from novel techniques, to be considered in this way.[6]

Medicinal products intended to be added to feed, and other compounds for which no medicinal claims are made, including mineral supplements and amino acids, are considered under the terms of Council Directive 70/524/EEC ('concerning additives in feeding-stuffs') and not under the Medicines Directives.[7] Under article 7.2.A(b) of amending Directive 84/587/EEC, an ingredient is acceptable if 'at the level permitted in

[3] K. N. Woodward, in 'Textbook of Applied and General Toxicology', ed. B. Ballantyne, T. Marrs, and P. Turner, Macmillan, Basingstoke, in press.

[4] Council Directive of 28 September 1981 on the approximation of laws of Member States relating to veterinary medicinal products (81/851/EEC). *Official Journal of the European Communities*, 1981, No. L317, 1.

[5] Council Directive of 28 September 1981 on the approximation of laws of Member States relating to analytical, pharmaco-toxicological and clinical standards and protocols in respect of the testing of veterinary medicinal products (81/852/EEC). *Official Journal of the European Communities*, 1981, No. L317, 16.

[6] Commission of the European Communities' 'The Rules Governing Medicinal Products in the European Community', Volume V, Veterinary Medicinal Products, EC, Luxembourg, 1989.

[7] Council Directive of 23 November 1970 concerning additives in feedingstuffs (70/524/EEC). *Official Journal of the European Communities*, 1970, No. L270, 840.

feeding stuffs, it does not adversely affect human or animal health or the environment nor harm the consumer by altering characteristics of livestock products'. Basically this means that active ingredients to be considered for inclusion into feed must satisfy the same criteria for human, animal, and environmental safety as their more conventional medicinal counterparts.

To gain market authorization within the Community, an active ingredient for inclusion in feed must be entered into either Annex I or Annex II of Directive 70/524/EEC. Entry into Annex I means that the compound in question must be made freely available throughout the EC. Compounds with a less complete data package may enter into Annex II as a transitory measure pending further information. If these data are both forthcoming and satisfactory, the ingredient in question will enter Annex I, but if the data show adverse effects or indeed are not supplied by a sponsor(s) the compound falls from the Annexes and effectively loses market authorization.

To be considered for Annex entry an applicant usually approaches its own national authority, which in the case of the UK is the VMD. Under these circumstances the application is considered like any other product licence application and a VPC opinion is sought. Should this be favourable, officials then guide the application through the EC procedures which take the form of the Standing Committee for Feeding Stuffs and an EC Expert Working Group. Advice on toxicological and other specialized areas is provided by the Scientific Committee on Animal Nutrition or SCAN and ultimately, if all is satisfactory, Annex entry is recommended.

4 Residues

When a drug is given to food-producing animals, the main area of concern is undoubtedly consumer safety arising from the presence of residues. Residues are the small amounts of the drug and its metabolites which remain in animal tissues at the time of slaughter or which are passed into other edible animal products such as eggs, milk, and honey as a result of treatment.

The first stage in assessing the safety of residues is the examination of a package of toxicity data submitted by the licence applicant, so that a toxicological profile of the drug can be established. The information is scrutinized so that a full picture of the biological properties of the compound can be built up and any particular adverse properties can be defined. This process is aided by carefully considering other relevant biological data in addition to information provided by toxicological tests. These will include information on absorption, biotransformation, and excretion in laboratory and target species, data from pharmacodynamic studies, and toxicity data from trials in the proposed target animals. Although the veterinary use of the drug may be new, it may have previously been employed in human medicine and valuable information

may be available from this source either in the form of adverse reaction reports or, more rarely, from epidemiological studies.

The simplest case to consider is that of a new molecule solely intended for use in veterinary medicine. An application for this will be accompanied by a series of toxicity reports generated in laboratory animals or in *in vitro* systems and in the target species, accompanied by the results of pharmacological studies, again generated in laboratory and target animals. If the drug is intended for use in food-producing animals, the range of toxicity data required will be extensive and may consist of acute and subacute studies, investigation into effects on reproduction and development, mutagenicity and carcinogenicity studies, and specialist studies to elucidate particular aspects of toxicity, *e.g.* neurotoxicity or teratology. The major goal is to identify a no-effect level from the data package.[8,9] From the no-effect level, an acceptable daily intake (ADI) can be calculated using an appropriate safety factor. The safety factor usually has a value of 100 but for severe forms of toxicity or where there is some degree of uncertainty about the toxicity or the no-effect level, a larger factor may be chosen.[10–12] The ADI, with a knowledge of food intake data, is then used to establish a Maximum Residue Level (MRL). Unfortunately, food intake data are difficult to generate and research is complicated by members of the population with extreme intakes of a particular food commodity such as those noted in ethnic or religious groups. Nevertheless, such studies are conducted, and the Ministry of Agriculture, Fisheries and Food publishes total diet information.[13] In practice, standard food intake data, which include large safety margins, are usually employed.

Once the MRL is established for the active ingredient, withdrawal periods can be determined. The withdrawal period is the time from cessation of dosing until the animal is acceptable for slaughter when the residues of the drug have fallen below the MRL. In order to identify a withdrawal period, groups of animals are given the drug as the intended proprietary formulation using the intended route of administration. It cannot be overemphasized that the ADI and the MRL apply to the active ingredient but the withdrawal period pertains to each of its formulations, as some may be designed to be fast acting and hence be rapidly cleared from the tissues while others may be slow release products and residues

[8] N. H. Booth in 'Veterinary Pharmacology and Therapeutics', 5th Edn., ed. N. H. Booth and L. E. McDonald, Iowa State University/Ames, 1982, p. 1065.
[9] K. N. Woodward, in 'Clinical and Experimental Toxicology of Anticholinesterases', ed. B. Ballantyne and T. Marrs, Butterworth, London, in press.
[10] E. J. Bigwood, *CRC Crit. Rev. Toxicol.*, 1973, **2**, 41.
[11] M. K. Perez, *J. Toxicol Environ. Health*, 1977, **3**, 837.
[12] G. Vettorazzi and B. Radaelli-Benvenuti, 'International Regulatory Aspects for Pesticide Chemicals', Vol. II, Tables and Bibliography, CRC Press, Boca Raton, Florida, 1982, p. 109.
[13] M. E. Peattie, D. H. Buss, D. G. Lindsay, and G. A. Smart, *Food Chem. Toxicol.*, 1983, **21**, 503.

may persist for considerable periods of time. Groups of dosed animals are then slaughtered so that the residue depletion can be monitored, and the time point when levels fall below the MRL identified. The withdrawal period is then specified as part of the licence requirements for the product in question. Eggs, milk, and honey present particular problems. Animals can be retained from slaughter using a suitable withdrawal period until drug residues decay to below the MRL but residues in eggs, milk, and honey do not deplete with time. Consequently, it may be necessary to discard these commodities until levels fall to or below the MRL.

It is of course of very little practical use to expend time and money on toxicological studies and the MRL procedures if withdrawal periods are ignored and residues then enter the food chain at unacceptable levels. There is an obvious need for a policing system. The so-called Residues Directive was published in the Official Journal of the European Communities in 1986 and was incorporated into UK legislation in 1988 in the form of the Animals and Fresh Meat (Examination for Residues) Regulations 1988.[14,15] These regulations make provision for the analysis of tissue samples and for the restriction of animals containing residues above official MRLs. These MRLs have yet to be established; they are currently under consideration by the CVMP's Working Group on Residues. Nevertheless, surveillance for residues in the UK is now extensive and responsibility for it lies with the VMD in collaboration with the State Veterinary Service. In 1989, some 40 000 samples were analysed and this figure is set to increase. As well as monitoring for residues above the MRL, the surveillance system is also a check for the presence of banned substances, including anabolic steroids and thyrostatic compounds. In addition to the residue sampling conducted by the State Veterinary Service National Surveillance Scheme, the Steering Group on Food Surveillance oversees a programme of surveillance through its Working Party on Veterinary Residues in Animal Products. The results of this work are considered by the Department of Health's Committee on Toxicity of Chemicals in Food, Consumer Products, and the Environment and where relevant by its sister Committees on mutagenicity and carcinogenicity, before being put to the VPC. This body then considers the data and any recommendations from the Department of Health's Committees before making its own pronouncements which in problem areas may involve modifying the MRL or the conditions of a product licence. The results and Committee deliberations are published in the form of a Food Surveillance Paper.[16,17]

[14] Council Directive of 16 September 1986 concerning the examination of animals and fresh meat for the presence of residues (86/469/EEC). *Off. J. Eur. Commun.*, 1986, No. L275, 36.

[15] Statutory Instruments No. 848 Agriculture. The Animals and Fresh Meat (Examination for Residues) Regulations 1988, HMSO, London, 1988.

[16] Anabolic, Anthelmintic, and Antimicrobial Agents. The Twenty-Second Report of the Steering Group on Food Surveillance. The Working Party on Veterinary Residues in Animal Products. Food Surveillance Paper No. 22, HMSO, London, 1987.

[17] Food Surveillance 1985 to 1988. Progress Report of the Steering Group on Food Surveillance (1988). The Twenty-Fourth Report of the Steering Group on Food Surveillance, HMSO, London, 1988.

There are some circumstances where an MRL cannot be identified. These present particular problems for both industry and for the regulatory agencies. The best, or perhaps worst example is when the active ingredient of interest is a mutagen or a genotoxic carcinogen.[18,19] In theory no no-effect level can be established for these effects[19] and so an ADI or MRL cannot be calculated. It has been suggested that the no threshold effect and the zero tolerance concepts are untenable,[20] and that various methods can be employed to assess the degree of risk from problem compounds.[21] However, in a world where consumer issues are uppermost in many people's minds, the practice of allowing compounds for which a no-effect level cannot be identified into the food-chain is unacceptable. Drugs containing such compounds are therefore generally reserved for non-food producing animals.

5 Future Prospects

There are numerous pressures which may ultimately shape the requirements of veterinary medicine licensing, both in the UK and in the European Community as a whole. These include pressures from individual consumers, from the so-called Green Lobby, and from industry. However, perhaps the most fundamental change will be that arising from ideas to create a European licensing agency. At present ideas in this area are perhaps not surprisingly vague but the European Commission has signalled its intentions to establish such an agency.[22,23] It is intended that this organization would bring together the market authorization of veterinary and human medicines in the Community and this route of authorization would become more widely used – as opposed to national licensing – after 1992. So far there are no detailed plans as to the location of the organization but it is hoped that more detailed ideas on its function, siting, and operation will be available in the early 1990s. However, it seems likely that the national licensing operations will need to be in existence for the foreseeable future, to cope with domestic situations and to provide a source of expertise for European bodies.

On the scientific front there are still major issues which need to be overcome in the safety assessment of veterinary drugs. The induction of antibiotic resistance in bacteria has been a contentious issue for some time

[18] W. Flam, in 'Chemical Safety Regulation and Compliance', ed. F. Homburger and J. K. Marquis, S. Karger, London, 1985, p. 1.

[19] F. C. Lu, 'Basic Toxicology, Fundamentals, Target Organs and Risk Assessment', Hemisphere Publishing, London, 1985, p. 81.

[20] A. Somogyi, 'Carcinogenic Risks. Strategies for Intervention', IARC Scientific Publications, No. 25, 1979, p. 123.

[21] M. L. Dourson, R. C. Hertzberg, R. Hartung, and K. Blackburn, *J. Toxicol. Ind. Health*, 1985, **1**, 33.

[22] Commission of the European Communities. Memorandum on the Future System for the Authorization of Medicinal Products in the European Community, III/B/6, April, 1989.

[23] Commission of the European Communities. Future System for the Free Movement of Medicinal Products within the European Community (Four Preliminary Draft Proposals), III/3603/90-EN, February, 1990.

with the concern that the use of antibiotic and antimicrobial drugs in veterinary medicine might lead to the emergence of resistant human pathogens. So far there is no firm evidence for this phenomenon.[3]

The recently formed Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) has begun to consider MRLs for residues of veterinary drugs in edible animal products. It is advised on toxicological and residue matters by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) which has now met three times to consider veterinary medicines. The deliberations of these prestigious bodies are bound to have an effect on both national licensing agencies and on discussions within the European forum. This will be particularly important if non-EC countries adopt Codex MRLs which differ from those chosen by the European Community as this situation would create obvious barriers to commerce and the possibility of export–import disputes would arise. There is an increasing need therefore for harmonization of acceptable residue levels to prevent situations of this type from arising.

It is possible to be certain of only one factor in the future. Consumer and political interest in food quality and safety will ensure that, both domestically and internationally, pressure will continue for stringent safety assessment of ingredients used in veterinary medicines so that the public can be reassured that animal produce is without risk to human health.

CHAPTER 9

Analysis of Pesticides at Low Levels in Drinking Water

KEITH M. MOORE

1 Introduction

The presence of pesticides in the aquatic environment has been known since the development of sensitive analytical methods for determining organochlorine insecticides back in the late 1950s.[1] A survey of organochlorine insecticides in selected raw and treated waters in England and Wales was reported in 1968.[2] Although organochlorine insecticides were detected during this survey it was concluded that the low levels found in drinking water would contribute only a very small portion of the total dietary intake of pesticides. Until recently there had been little monitoring of the concentrations of pesticides in drinking water, because the very low levels present were not considered to be adverse to health. However, in the 1980s with the impetus of the EC Drinking Water Directive, the monitoring of pesticides became more common. From 1 January 1990 the routine monitoring of pesticides in drinking water has been required of water undertakers (Water Supply plcs and companies) by English and Welsh law under the Water Supply (Water Quality) Regulations 1989,[3] made under the Water Act 1989.

The introduction of the Water Supply Regulations has provided a stimulus for the development of analytical methods which are sufficiently sensitive and accurate to meet the requirements of the regulations, and of methods that are cheaper and easier to use than previous analytical

[1] F.M. Middleton and J.J. Lichtenberg, *Ind. Eng. Chem.*, 1960, **52**, 99A.
[2] B.T. Croll, 'Pesticide Residues in Water-II. Levels of Organochlorine Insecticides in Selected Waters in England and Wales', Water Research Association Report TP 62, 1968.
[3] Water Supply (Water Quality) Regulations 1989, Statutory Instruments 1989 No. 1147, HMSO, London, 1989.

methods. Since the Water Supply Regulations and their supporting documents define the analytical requirements for drinking water in the UK, it is appropriate to review them in some detail before discussing their implications for analytical methodology.

2 The Regulations Applying to Pesticides in Drinking Water

2.1 The Development of Regulations

Prior to 1980 the view accepted in the UK was that of the World Health Organization, *i.e.* that drinking water generally makes only a minor contribution to the daily intake of pesticides, but that the contamination of water by pesticides should be prevented as far as possible.[4]

In 1980 the European Community adopted the Directive relating to the Quality of Water intended for Human Consumption (commonly known as the EC Drinking Water Directive).[5] There are sixty-two parameters (organoleptic, physical, chemical, and microbiological) specified in the Directive. These include parameter 55, 'Pesticides and Related Products', which are defined as insecticides, herbicides, fungicides, polychlorinated biphenyls (PCBs), and polychlorinated terphenyls (PCTs). However, this definition is not precise and it is unclear whether other classes of pesticide (*e.g.* rodenticides or even growth regulators) and metabolites of pesticides are included. Maximum admissible concentrations (MACs) and occasionally guide levels were set for the parameter 55 compounds. The MACs for individual and total pesticides are 0.1 and 0.5 $\mu g l^{-1}$ respectively. It has been claimed that the MACs for pesticides were based on the detection limit for an analytical method for organochlorine insecticides at the time the Directive was formulated.[6] In general, it seems the rationale behind parameter 55 was that pesticides should not be present in drinking water.[7] The MACs were set by the Directive but insufficient guidance was given on the analytical performance required.

The Directive was implemented in England and Wales in 1982 by the issue of a circular by the Department of the Environment (DoE) and the Welsh Office.[8] However, two reservations concerning the MACs for parameter 55 were expressed in a Water Guidance letter issued by the DoE and the Welsh Office in 1986.[9] First, the MACs were not set using a toxicological basis, and secondly it was not possible to monitor compliance for many of the pesticides since analytical methods with sufficiently low

[4] European Standards for Drinking Water, WHO, 1970.
[5] EC Directive 80/778/EEC. The Quality of Water Intended for Human Consumption. *Off. J. Eur. Commun.*, No. L229/11-29.
[6] B.G. Johnen, *Pestic. Outlook*, 1989, **1**, 9.
[7] G. Premazzi, G. Chiaudani, and G. Ziglio, 'Scientific Assessment of EC Standards for Drinking Water Quality', Commission of the European Communities, Luxembourg, 1989.
[8] Joint Circular, Department of the Environment 20/82, Welsh Office 33/82.
[9] Water Guidance Letter, Department of the Environment/Welsh Office WP10/1986.

detection limits were not available. With the first of these reservations in mind the Water Guidance letter contained a list of pesticides and their associated health advisory values (*i.e.* maximum acceptable drinking water concentrations based on toxicological considerations) set by the DoE and Department of Health and Social Security. The letter also gave advice on the frequency of monitoring, available analytical methods, and the pesticides to monitor. Much of this was incorporated in the Water Supply Regulations and a subsequent Water Guidance letter published in 1989.[10]

2.2 The Water Supply Regulations

The Water Supply (Water Quality) Regulations 1989 were enacted under the Water Act 1989 among other reasons to enforce the EC Drinking Water Directive in English and Welsh Law. There are also similar regulations for Scotland (enacted in 1990) and Northern Ireland (currently being produced) to enforce the EC Drinking Water Directive throughout the UK. The Water Supply Regulations define pesticides and related products and set the same MACs as the EC Drinking Water Directive. In addition, the Water Regulations and supporting documents prescribe sampling points and sampling frequencies and specify the requirements for analytical performance.

2.3 The Requirements of the Water Supply Regulations

The requirements of the Water Supply Regulations in relation to pesticides in drinking water are as follows.

2.3.1 Sampling

Samples taken to monitor compliance with the Water Supply Regulations must be taken and stored such that they are: representative of the quality of water throughout the water supply zone at the time of sampling; not contaminated during sampling and analysis; kept under conditions which ensure that there is no material change in the concentration of pesticides; and kept for the minimum time possible (*i.e.* analysed as soon as practicable).

The number of samples to be taken is specified in relation to water supply zones. A water supply zone is an area designated by a water undertaker in which not more than 50000 people reside. The standard sampling frequency to be applied at consumers' taps is four samples per annum per water supply zone. However, if in three successive years the water samples taken contained less than 0.05 $\mu g l^{-1}$ of a single pesticide, and less than 0.25 $\mu g l^{-1}$ of total pesticides, and the water undertaker is sure that the concentrations will not rise in the subsequent year, then a

[10] Water Guidance Letter, Department of the Environment/Welsh Office WP18/1989.

reduced sampling frequency is allowed. The reduced sampling frequency is one sample per annum per water supply zone. If the MACs for single or total pesticides have been exceeded then an increased sampling frequency is required. The increased sampling frequency is either 12 or 24 samples per annum per water supply zone, depending on whether the water supply zone supplies more than 35 000 people or 7000 $m^3 d^{-1}$.

A water undertaker may sample from a point other than a consumer's tap, if the Secretary of State can be satisfied that analysis of a sample from this point will produce data which are unlikely to differ from data produced by analysis of a sample from a consumer's tap. Different sampling frequencies apply to these sampling points.

2.3.2 *Analysis*

The Water Supply Regulations state that samples should be analysed as soon as possible after sampling, by or under the supervision of a person competent to perform that task, using suitable equipment, and using analytical systems and methods which are capable of establishing (within acceptable limits of deviation and detection) whether the MACs have been exceeded. Also, the laboratory at which the samples are analysed should have a system of quality control that is subjected from time to time to checking by a person who is not under the control of the laboratory or the water undertaker and is approved for that purpose by the Secretary of State.

The document 'Guidance on Safeguarding the Quality of Public Water Supplies'[11] (referred to here as the Guidance Notes) gives further advice on what is required of analytical methods. This states that the analytical method should determine the total parameter concerned (*i.e.* dissolved, colloidal, and particulate-associated pesticide, plus pesticide in any other chemical form, *e.g.* both ester and free acid forms of phenoxy-acid herbicides). It also states that for most parameters covered in the Water Supply Regulations the performance required of analytical systems is:

(i) A maximum tolerable total error of individual results not exceeding one tenth of a prescribed concentration or 20% of the result, whichever is the greater;
(ii) A maximum tolerable total standard deviation of individual results not exceeding one fortieth of the prescribed concentration or 5% of the result, whichever is the greater;
(iii) A maximum tolerable systematic error (or bias) of individual results not exceeding one twentieth of the prescribed concentration or 10% of the result, whichever is the greater;
(iv) A limit of detection (4.645 times the within-batch standard deviation

[11] Guidance on Safeguarding the Quality of Public Water Supplies, Department of the Environment/Welsh Office, HMSO, 1989.

of results for blanks) equal to (or presumably, if possible, less than) one tenth of the prescribed concentration;

(v) A recovery not significantly less than 95% or significantly greater than 105%.

The definitions for terms such as total error and systematic error are given by Hunt and Wilson.[12]

Table 1 shows what these performance requirements would mean in the case of individual pesticides. What the requirements would mean in terms of the total pesticide parameter (defined in the guidance notes as the sum of the detected concentrations of individual pesticides) is more difficult to define, since it depends on the number of pesticides monitored. However, in the guidance notes it is recognized that the level of performance in Table 1 is not possible for many pesticides using currently available analytical methods. Therefore the guidance is that the best currently available analytical methods should be used. The methods published by the Standing Committee of Analysts are given as examples of these.

Table 1 *Ideal performance characteristics required of analytical methods*

Performance characteristic	*Values of performance characteristic allowed*	
	Analytical result $\leq 0.05\ \mu g l^{-1}$	*Analytical result* $>0.05\ \mu g l^{-1}$
Maximum tolerable total error	$0.01\ \mu g l^{-1}$	20% of result
Maximum tolerable total standard deviation	$0.0025\ \mu g l^{-1}$	5% of result
Maximum tolerable systematic error	$0.005\ \mu g l^{-1}$	10% of result
Limit of detection	$0.01\ \mu g l^{-1}$	$0.01\ \mu g l^{-1}$
Recovery	>95% but <105%	>95% but <105%

There are a large number of pesticides used both agriculturally and non-agriculturally in the UK and consequently it is not feasible to monitor all of them. Guidance is therefore needed on which should be monitored. The Guidance Notes state that each water undertaker is required to develop a monitoring strategy for pesticides based on the likely risk of particular pesticides being present in the water source serving the zone. In developing a monitoring strategy water undertakers are advised to assess, as far as practicable, which pesticides are used in significant amounts within the catchment area, and whether any of these pesticides are likely to reach a water source in a catchment, based on their properties and method of use and on local catchment knowledge. The Water Guidance letter WP

[12] D. T. E. Hunt and A. L. Wilson, 'The Chemical Analysis of Water–General Principles and Techniques', 2nd Edn., The Royal Society of Chemistry, London, 1986.

18/1989[10] gives information on the properties and usage rates of some pesticides likely to enter water, to help the water undertaker develop a monitoring strategy.

2.3.3 Analytical Quality Control

The importance of analytical quality control for checking and helping to improve the performance of analytical systems is recognized in the Guidance Notes.[11] For internal quality control, laboratories are expected to carry out the following procedures:

(i) Use an analytical method capable of achieving the required accuracy, and which is written down unambiguously and in sufficient detail.
(ii) Estimate the within-laboratory total standard deviation of individual analytical results for blanks, standard solutions, samples, and spiked samples having concentrations over the range of interest, over at least five batches on five separate days. Each estimate of total standard deviation should have at least ten degrees of freedom. The recovery of added spikes of the pesticide from typical samples should also be assessed to check certain sources of bias.
(iii) Set up and maintain a quality control chart to check the routine performance of the analytical system. As a minimum the control analysis should be of a standard solution with a concentration close to the prescribed concentration, *i.e.* 0.1 $\mu g l^{-1}$ in the case of pesticides.

Laboratories are also expected to participate in external quality control schemes where available, which involve the distribution of check samples. Such a check sample quality control scheme is available for a variety of pesticides from the WRc Service Aquacheck. Some results obtained by Aquacheck are presented in Table 2. They show that there can be wide variations in the accuracy of analytical results when analysing pesticides at low levels in water. Some analytical results were in error by more than 100%. Other inter-laboratory studies have also shown that some pesticide analytical results can be very inaccurate,[13] and therefore it is important to have good internal quality control procedures.

3 Analytical Methods for Pesticides in Water

The discovery of organochlorine insecticides in raw waters was made possible by the development of gas chromatography (GC) in the 1950s. Methods similar to those used in the 1950s employing solvent extraction and gas chromatography are still used, but there have been significant developments in analytical techniques in recent years.

[13] L.A. Norris, *Weed Sci.*, 1986, **34**, 485.

Table 2 *Summary of results from AQUACHECK pesticide samples*

Pesticide	*Spiked concentration* ($\mu g l^{-1}$)	*No. of labs reporting*	*% of laboratories reporting*	
			Values in error by ≥50% of spiked value	*Values in error by ≥100% of spiked value*
Endrin	0.034	27	15	0
Dieldrin	0.056	31	16	3
Aldrin	0.049	33	15	3
pp'-DDT	0.058	29	38	3
Lindane	0.044	33	12	0
Simazine	0.084	15	0	0
Atrazine	0.078	15	13	7
Propazine	0.031	16	25	13
Azinphos methyl	0.063	3	33	33
Dichlorvos	0.072	4	0	0
Fenitrothion	0.065	5	20	20
Malathion	0.041	8	63	25
Mevinphos	0.054	3	33	17
Chlorfenvinphos	0.026	6	33	17

3.1 Official Analytical Methods

The official source of analytical methods for pesticides in water in the UK is the Standing Committee of Analysts (SCA). The methods currently available were all published before the introduction of the Water Supply Regulations. However, some of the methods are capable of achieving detection limits below 0.1 $\mu g l^{-1}$. Table 3 lists current SCA methods for pesticides and their detection limits. There are also a large number of SCA analytical methods for pesticides in draft form at the time of writing this chapter. Although limits of detection below 0.1 $\mu g l^{-1}$ are possible with some SCA analytical methods, problems with interferences have been encountered, for example with the phenoxy acid-herbicides.[14] Interferences can be encountered as many existing SCA methods use non-specific detection techniques.

The current SCA analytical methods do not meet all the requirements for analytical performance described in Table 1. However, the Guidance Notes state that as current methods for pesticides cannot meet these requirements, the best currently available analytical methods (*e.g.* the SCA methods) should be used.

[14] A. Waggott, Proceedings of COST 641, Workshop held at Water Research Centre, Medmenham, UK, 23–24 November 1988, ed. B. Crathorne and G. Angeletti, Commission of the European Communities, Water Pollution Research Report II, 1989.

Table 3 *Currently available SCA methods for pesticides*

Pesticides covered	*Reference*	*Detection limits quoted* ($\mu g l^{-1}$)
Organochlorine insecticides	*a,b,c*	0.003–0.015
Total PCBs	*a,b,c*	0.1
Organophosphorus insecticides	*d,e*	0.03–0.4
Chlorophenoxy acidic herbicides	*f*	0.004–0.1
Trichlorobenzoic acid	*f*	0.0005
Chlorophenols	*f*	0.02–0.2
Triazines	*f*	0.025
Glyphosate	*f*	0.08
Carbamates	*g*	0.02–0.29
Thiocarbamates	*g*	0.48 as CS_2
Urons	*g*	<5
Diquat & Paraquat	*h*	0.02
Pyrethrins	*i*	0.24
Permethrin	*i*	0.06

[a] Organochlorine Insecticides and PCBs in Water, Standing Committee of Analysts, HMSO, London, 1978.
[b] The Determination of Organochlorine Insecticides and PCBs in Sewage, Sludges, Muds and Fish 1978, in Water (an addition), Standing Committee of Analysts, HMSO, London, 1984.
[c] Chlorobenzenes in Water, Organochlorine Insecticides and PCBs in Turbid Waters, Halogenated Solvents and Related Compounds in Sewage Sludge and Waters, Standing Committee of Analysts, HMSO, London, 1985.
[d] Organophosphorus Pesticides in River and Drinking Water (tentative method), Standing Committee of Analysts, HMSO, London, 1980.
[e] Organophosphorus Pesticides in Sewage Sludge, in River and Drinking Water, Standing Committee of Analysts, HMSO, London, 1985.
[f] Chlorophenoxyacidic Herbicides, Trichlorobenzoic Acid, Chlorophenols, Triazines and Glyphosate in Waters, Standing Committee of Analysts, HMSO, London, 1985.
[g] The Determinaton of Carbamates, Thiocarbamates, Related Substances and Ureas in Water, Standing Committee of Analysts, HMSO, London, 1987.
[h] Determination of Diquat and Paraquat in River and Drinking Water, Spectrophotometric Methods (tentative), Standing Committee of Analysts, HMSO, London, 1987.
[i] Pyrethrins and Permethrin in Potable Waters by EC-GC, Standing Committee of Analysts, HMSO, London, 1981.

3.2 Developments in Analytical Methodology to Meet the Requirements of the Water Supply Regulations

The majority of analytical methods currently used are based on solvent extraction followed by chromatography and detection with a non-specific detector. There have been developments in both the extraction and detection steps of the conventional pesticide analytical methods, and some new approaches to pesticide analysis have recently emerged, involving automation and/or analytical methods using different principles (*e.g.* immunoassays).

3.2.1 Developments in the Extraction of Pesticides

A development which can improve the performance of the extraction step

in pesticide analysis is the use of solid-phase extraction (SPE). This technique involves extraction of water samples using a solid sorbent material, such as amberlite XAD resin and, more recently, bonded silica sorbents in cartridges. For many pesticides SPE achieves better extraction efficiencies than solvent extraction.[15] SPE also offers other advantages over solvent extraction, including: greater sample throughput rate, ease of use, less use of potentially harmful solvents, potential for automation, and greater selectivity.

Bellar and Budde[15] reported that the precision was worse for SPE than for solvent extraction, but the pesticides for which precision was poor were those for which the recoveries were low. Probably if more suitable stationary phases were used for these pesticides, then better precision would be obtained.

3.2.2 Developments in the Detection of Pesticides

Analytical methods using non-specific detectors, such as GC–ECD, are satisfactory for pesticides in drinking water when monitoring compliance with toxicologically based water quality standards since such standards are invariably considerably higher than 0.1 $\mu g l^{-1}$. However, interferences may be encountered when using these methods to monitor compliance with the 0.1 $\mu g l^{-1}$ MAC. When an analytical method is being used to monitor one or two pesticides it is normally easy to choose a chromatographic column which separates the pesticides from the interferences. However, when larger numbers of pesticides are being analysed in one chromatographic run (*i.e.* in a multi-residue method) this becomes more difficult. The problem encountered with interferences when using a nitrogen phosphorus detector (NPD) for analysing 'dirtier' water is illustrated in Figure 1.

The problem of interferences can be solved in two ways. One approach is to eliminate the interferences using a clean-up technique. This approach has been used for the analysis of triazine herbicides[16] and phenoxy-acid herbicides.[17] An ion-exchange clean-up technique was used in both of these methods and it appears to have been successful, as can be seen from the HPLC chromatograms shown in the respective papers. Other types of clean-up can be used, such as normal phase column chromatography on Florisil, silica gel, or alumina. The disadvantage of this approach is that the pesticides to be determined must be chemically similar to each other but different from the interferences. This means that the number of pesticides that can be determined by any one method is limited.

Another approach for avoiding interference is to use a highly selective detector such as a mass spectrometer. This approach is very successful as

[15] T.A. Bellar and W.L. Budde, *Anal. Chem.*, 1988, **60**, 2076.
[16] M. Battista, A. DiCorcia, and M. Marchetti, *Anal. Chem.*, 1989, **61**, 935.
[17] A. DiCorcia, M. Marchetti, and R. Samperi, *Anal. Chem.*, 1989, **61**, 1363.

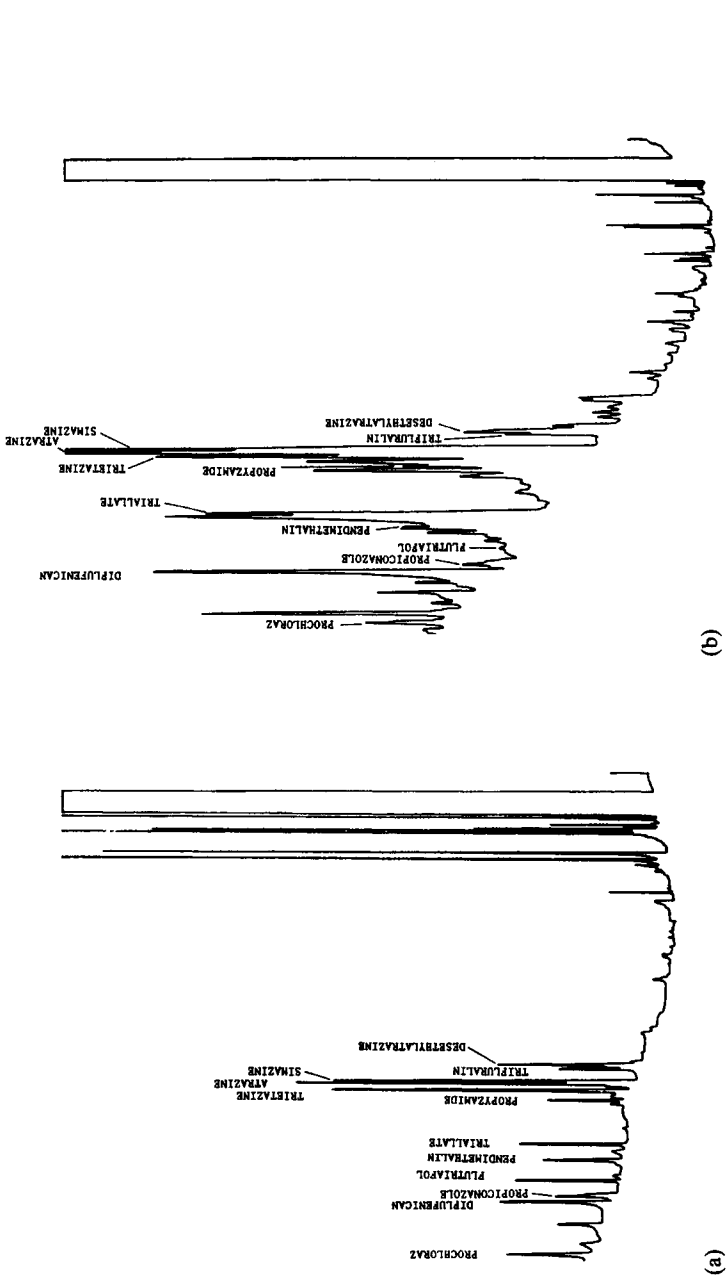


Figure 1 *GC–NPD chromatograms of* (a) *borehole derived drinking water spiked at* 0.1 $\mu g l^{-1}$ *with various nitrogen-containing pesticides;* (b) *river water spiked at* 0.1 $\mu g l^{-1}$ *with various nitrogen containing pesticides: pH 8, dichloromethane extracts reconstituted in* 100 µl *of ethyl acetate;* 1 µl *on-column injection onto* 30 m *DB5 (J&W) column held at* 60 °C *for* 3 min, *then programmed to* 300 °C *at* 8 °C min^{-1}

can be seen in the gas chromatograph–mass spectrometer (GC–MS) trace in Figure 2. Mass spectrometry is a selective and sensitive detection technique which, with the different ionization modes now available (thermospray, chemical ionization, *etc.*), can be used to determine the majority of pesticides. The disadvantage of mass spectrometry is that it is more costly than other detectors and consequently it is not so readily available (although most water undertakers have access to one). However, when less costly non-specific detectors are used it is advisable to confirm significant results using mass spectrometry.[18]

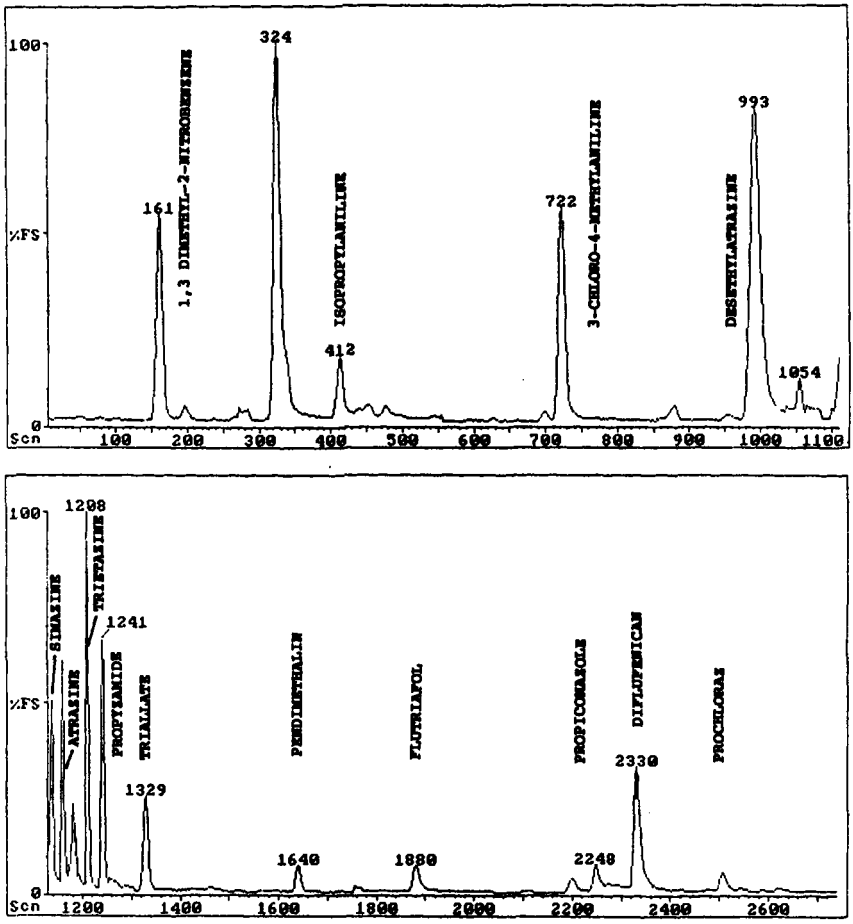


Figure 2 *GC-MS selected ion monitoring total ion current chromatogram of borehole water spiked at* 0.1 $\mu g l^{-1}$ *with various nitrogen containing pesticides: pH 8,* C_{18} *solid-phase extract reconstituted in* 100 μl *of ethyl acetate;* 1 μl *on-column injection onto* 30 m *DB1 (J&W) column held at* 60 °C *for* 3 min, *then programmed to* 300 °C *at* 8 °C min^{-1}

[18] U. Oehmichen, F. Karrenbrock, and K. Haberer, *Gewaesser. Wasser Abwasser,* 1989, **106**, 110.

3.2.3 *Developments in Producing Lower Cost and Automated Methods*

The Water Supply Regulations require a considerable number of pesticide analyses to be carried out, and so there is a need for low cost and automated methods. There have been various approaches to this including thin-layer chromatography (TLC), on-line solid-phase extraction, and immunoassay.

Thin-layer chromatography (TLC) is a cheap semi-automatable technique which can achieve detection limits just below 0.1 $\mu g l^{-1}$ for some pesticides. However, the precision of this technique is poorer than that for high-performance liquid chromatography or gas chromatography; Zietz *et al.*[19] give relative standard deviations of 15–20%. TLC also has the disadvantage that it is prone to interferences as the detectors commonly used are not very specific. TLC is therefore probably best used as a screening technique for relatively clean samples.

On-line solid-phase extraction is a technique using small cartridge HPLC columns to concentrate the pesticides and then a separate analytical column for on-line HPLC analysis (*e.g.* Hamann and Kettrup[20]). This technique can be totally automated and achieve good precision. However, it limits the analytical method to pesticides which have very similar chemical properties.

Immunoassay is a recent development in pesticide analysis, which uses different principles from conventional chemical analysis. The basis of the technique is that an antibody is used to bind selectively a particular pesticide. The pesticide can then be detected in a number of different ways. One common approach is to use an enzyme-labelled pesticide to compete with the pesticides in the sample for the free antibody sites and then to use the enzyme in a reaction to produce a colour change. This procedure is known as an enzyme-linked immunosorbent assay (ELISA).

Immunoassay is a technique which is relatively cheap to use, can be automated, and often has high specificity.[21] However, immunoassays have disadvantages: they usually determine one pesticide per assay, they have high development costs (especially associated with the production of antibodies), and there are relatively few commercially available immunoassays for pesticides. The low cost of the ELISA approach means that it is best used as a technique for screening large numbers of samples prior to the use of more conventional techniques (preferably mass spectrometry) to confirm significant results.

4 Conclusions

The Water Supply Regulations are stringent with respect to the perform-

[19] E. Zietz, I. Ricker, and G. Arendt, *Gewaesser. Wasser Abwasser*, 1989, **106**, 136.
[20] R. Hamann and A. Kettrup, *Chemosphere*, 1987, **16**, 527.
[21] F. Jung *et al.*, *Pestic. Sci.*, 1989, **26**, 303.

ance required of analytical methods for monitoring compliance with the MACs for pesticides. For many pesticides this performance is difficult to achieve using currently available analytical methods. Inter-laboratory quality control studies have shown that there can be large inaccuracies in pesticide analytical data. It is therefore important that analytical methods are thoroughly tested to assess their performance, and quality control procedures are used to check that the required quality of analytical data is maintained.

Some currently available analytical methods which employ non-specific detectors can have problems with interferences. When using these methods it is advisable to confirm positive results using mass spectrometry.

In principle, the Water Supply Regulations require a considerable number of pesticide analyses to be carried out. Some recent developments in pesticide analysis which help make pesticide analyses cheaper or faster are solid-phase extraction and immunoassay. Solid-phase extraction is becoming more widely used because among its advantages it is quicker, easier to automate, and uses significantly smaller volumes of potentially harmful solvents than solvent extraction. Immunoassay appears to be a promising technique for the screening of large numbers of samples because once developed it is cheap to run, and quick and easy to automate, but it is advisable to confirm significant results using a conventional analytical technique, preferably based on mass spectrometry.

Acknowledgements

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CHAPTER 10

Unwanted Flavours in Foods

J. DAVID HENSHALL

1 Introduction

In today's complex food chain starting on the farm, the fishing boat, or even deep in the tropical jungle and ending on the consumer's dining table, there is a prime requirement for the processor to provide a product of safe and consistent quality which has strong consumer appeal. Three main attributes are carefully controlled, these being colour, texture, and flavour.

The raw materials of the food are largely of natural origin and are subject to variation in their characteristics. The art or skill of the food scientist and technologist is to control the formulation and processing of raw material to produce the required products. However, things do go wrong!

Flavour problems in a wide range of processed foods, including 'fresh produce', are increasing. The incidence of these outbreaks is still very low when the total volume of food passing through the distribution system is considered and the question which concerns those who supply high quality products is 'Why is this so?'. Either there is (i) a real problem, (ii) the consumer's expectations are higher than previously when variations in quality attributes were more tolerated, or (iii) consumers are more prepared to complain. Whatever the cause, there has been increased interest in the analysis of off-flavours and taints.

Off-flavours in food have always been with us and historically these have been caused by microbial or fungal contamination, or oxidative deterioration. Sometimes these strong flavours have been welcomed and enhanced and over many years have resulted in products which are now considered to be delicacies – the same flavour in another food is considered to be objectionable. In earlier times when food storage was a primitive 'process', these off-flavours were often disguised by the use of spices. The scene today is different – the consumer expects, and rightly so, food which is safe to eat and exhibiting good organoleptic properties. Therefore, when one of the major attributes such as flavour is not acceptable or just plain objectionable, the food supplier wishes to know what happened and why.

Food processors are often warned about a taint problem by consumers returning products through a retailer or by their making direct approaches to the company. Often it is possible to gain an idea of the severity of the problem by the numbers of complaints and the written comments which are received. Sometimes the geographical area from which the complaint arises can provide useful information if there is more than one notification – perhaps contamination occurred *en route* to the area or while the product was stored in a particular distribution centre. Even so, it is unwise to rely on consumers alone for a precise characterization or description of the taint – a trained tasting panel should be used. It is of great importance to use an expert panel to detect and describe the taint because for any chemical, in a human population, there will exist a wide range of sensitivities. Therefore in a panel, there should be people with sensitivities greater than those of the population mean.

In the past thirty years, many new synthetic chemicals have been introduced into the environment and some of these have entered the food chain and can be shown to occur in raw materials either as non-degraded compounds or metabolites. Today, both processed and fresh foods are affected by flavours variously described as earthy, catty, solvent-like, painty, mouldy, faecal, metallic, or cardboardy. These flavours may be introduced into the food or the packaging material by absorption from many sources including the environment in which food has been stored, grown, or processed. The environment in this context means soil, air, and water. Many of these compounds are man-made and it is these which cause some of the more severe flavour problems and about which precautions can be taken to reduce the risk of food contamination.

The compounds of interest in identifying a food as well as providing subtle differences to products of similar origin are the volatile components. Foods contain many hundreds of such compounds which either singly, or in combination, define precisely the identity of an acceptable food product and contribute to human or animal consumer preferences between different brands, products, or varieties. It is also the volatile compounds which are almost always responsible for the off-flavours which arise from contamination or chemical, biochemical, or microbiological transformation.

2 Analytical Methods

Mixtures of food flavour volatiles are invariably separated by gas chromatography (GC), almost always followed by mass spectrometry for the identification of particular compounds. Before analysis, however, there is the problem of extraction of the flavour components from the food matrix in a suitable form and concentration for GC analysis. The extraction stages usually employ solvent extraction, distillation, trapping of headspace volatiles on to an absorbent, or a combination of these techniques. The range of these combinations in the published literature is large, and the problem

is that each process is likely to produce different flavour profiles on analysis.

A typical GC trace of the volatile components from yeast is shown in Figure 1 and the complexity of the mixture can be seen.[1] The tainting compound in the sample was indole, eluting at 32.5 minutes. This was confirmed by mass spectrometry; the mass spectrum is shown in Figure 2. This illustrative sample is unusual in that the quantity of contaminant is large. A useful technique to narrow the selection of a peak and to relate it to the occurrence of a particular flavour is to sniff the effluent gas from the chromatograph.[2] The processes of extraction and concentration of flavour components have been the subject of many studies and it is known that each technique has advantages and disadvantages. A good example is that of sample/solvent co-distillation; the heating process may cause artefact formation and so the components of the volatile mixture should be compared with the results obtained by another technique, for example closed loop stripping.[3]

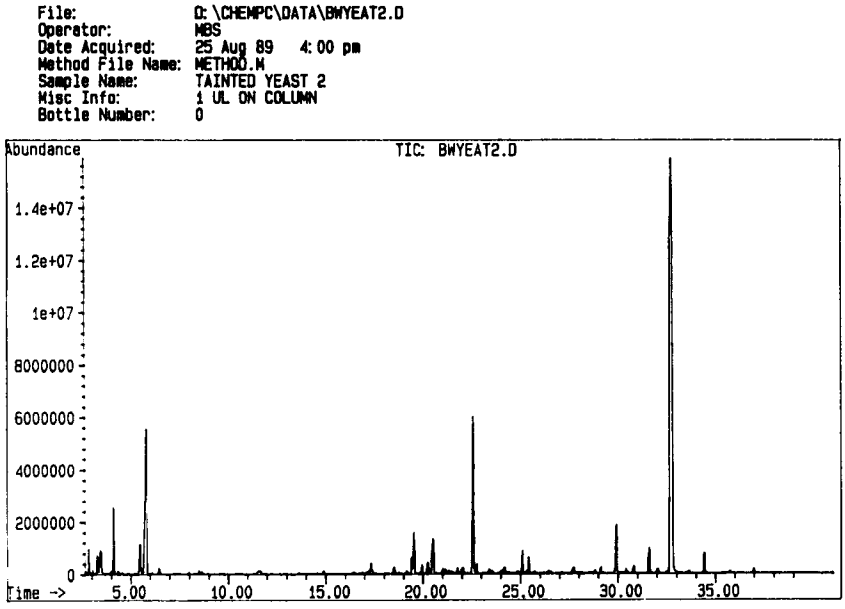

Figure 1 *GC trace of volatiles from a tainted sample of yeast*

[1] M. B. Springett, personal communication, 1990.
[2] N. M. Griffiths and D. G. Land, *Chem. Ind. (London)*, 1973, 904.
[3] K. Grob, Jr. and F. Zurcher, *J. Chromatogr.* 1976, **117**, 285.

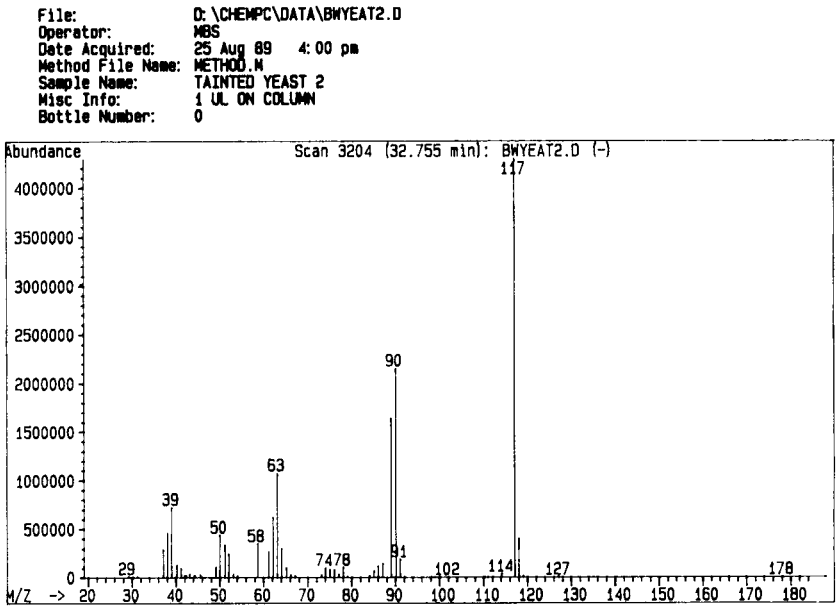

Figure 2 *Mass spectrum of indole, originating from a tainted sample of yeast*

3 Sources of Taints and Off-flavours

Taint is the term which is used to describe alien flavours in foods which come from outside sources and are perceived by a taster as being unpleasant. Generally speaking, the chemicals which cause taint are present in very low concentrations and are detected because of the low threshold values. These are nearly always at the parts per billion ($\mu g\ kg^{-1}$) level. Two consequences arise therefore. The first is that quantitation is difficult and requires sophisticated techniques and instrumentation to extract such small quantities from a complex and variable matrix. Secondly, in practice in instances where tainting occurs toxicity is not usually a problem for the consumer. (The latter observation obviously excludes malicious contamination which is quite another matter.) 'Off-flavour' is another term used to decribe food that does not have the expected or required flavour. This term should be used to describe those flavours which arise from microbiological activity or deteriorative processes such as oxidation and rancidity. A combination of sensory and chemical analysis should be used to trace the source of a problem – the human used as a detector is often more sensitive than chemical analysis, but because tainting chemicals behave differently in different foods, identification by chemical methods should always be undertaken.

Since the publication of the seminal article by Goldenberg and Matheson,[4] much experience has been gained by food analysts in this most

interesting area of food chemistry. A further survey was that written by Whitfield,[5] and an important work edited by Charalambous[6] has recently been published. The number of taint problems and descriptions of techniques referred to in these references indicates the magnitude and complexity of describing definitively the cause of an outbreak of off-flavours in a foodstuff. Coupled with this is the knowledge that, following the identification of a problem, the solution is always required urgently because the distributor and manufacturer have to put recall procedures very quickly into effect. It may be that a product has been tampered with and the offending chemical or chemicals may be toxic – in any case, responsible companies want an urgent answer in order that the problem is not repeated.

Various descriptions of off-flavours or taints have been defined. Experience has shown that the chlorophenols, such as 6-chloro-2-methylphenol, are responsible for disinfectant type flavours while the chloroanisoles cause mouldy and musty effects.[7,8] The isomer of the phenol is important; for example, 4-chloro-2-methylphenol has a taste threshold of $3\ mg\,kg^{-1}$ in biscuits and $0.12\ mg\,l^{-1}$ in water. For 6-chloro-2-methylphenol, the values are $5 \times 10^{-5}\ mg\,kg^{-1}$ in biscuits and $8 \times 10^{-5}\ mg\,kg^{-1}$ in water. To produce a disinfectant taint in biscuits requires $1 \times 10^{-4}\ mg\,kg^{-1}$ of the latter compound. Goldenberg[4] stated that when biscuits were exposed for 10 min to air at 300 yards from a herbicide factory, $0.08\ mg\,kg^{-1}$ of mixed chloro-2-methylphenols could be found in the biscuits, resulting in what was described as a 'gross contamination producing an offensive smell and taste'.

Very often a cause of food contamination by chlorophenols is from the water which is used in processing, either as an ingredient or as a processing aid. Formation of chlorophenols can occur by the use of heavily chlorinated water reacting with the food or by chlorinating certain types of mains water, for example those derived from peaty sources. In the former case, chlorine can react spontaneously with simple phenols, while in the latter the problem has occurred industrially when the chlorinated water has been used for steam generation in high-pressure boilers. A description often used in food off-flavour terminology is 'musty' and this sometimes arises from water. Maga[9] identified several molecules; geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol), 2-methylborneol (2-methylisoborneol), and an alkoxypyrazine (2-methoxy-3-isopropylpyrazine) as causing this effect. The odour-producing properties of these compounds vary according to the concentration. They are probably due to algal blooms in the water and indicate the importance of monitoring raw water quality in food processing operations,

[4] N. Goldenberg and H. R. Matheson, *Chem. Ind. (London)*, 1975, 551.
[5] F. B. Whitfield, *CSIRO Food Res. Q.*, 1983, **43**, 96.
[6] 'Flavours and Off-flavours', ed. G. Charalambous, Elsevier, Amsterdam, 1990.
[7] R. L. S. Patterson, *Chem. Ind. (London)*, 1972, 609.
[8] C. Engel, A. P. de Groot, and C. Weurman, *Science*, 1966, 270.
[9] J. A. Maga, *Food Rev. Int.*, 1987, **3** (3), 269.

expecially during warm weather. The use of algaecides to prevent algal growth is not to be recommended. Some have been shown to contain 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol, and Whitfield[5] cited a case where water treated in such a manner leaked from a refrigeration plant into process water and caused difficulties.

Until some years ago, the use of phenolic resins or paints in food processing operations gave rise to problems – not only of phenolic taints but also hydrocarbon-type flavours. It is now common practice – if not universal – to test all materials which come into contact with food or which may be used in the construction of food factories and handling facilities for their liability to taint food, and therefore contamination from this source is now, happily, rare.

Pesticides have been implicated, and proved, to be the source of phenolic taints.[5] Episodes from this source still occur and problems can be found when treated timber comes into contact with or in proximity to food. The wood pulp from which packing cases are made, wooden pallets, paper sacks, and adhesives can all contain residual fungicides, and contamination from these sources may occur; the offending flavour is usually phenolic. An interesting account of a sporadic taint problem in soft drinks by contamination from this source is given by Whitfield.[5] During two consecutive summers, an Australian soft drink manufacturer suffered from random outbreaks of a medicinal taint in his products. It was shown that the off-flavour was not in the product prior to canning and that the problem occurred only in a few cans at any one time. The compound causing the off-flavour was not identified in the early stages of the investigation. The source of the taint was eventually identified as arising from an agrochemical factory nearby. It was believed that the cause was 6-chloro-2-methylphenol which was carried by the wind to the soft drinks plant and that the material was absorbed by the can lacquer where cans were partially exposed at the edge of the pallets. The cans could be reclaimed by restoving but the cardboard spacers on the pallets were destroyed because they could have provided a continuing source of taint. At the time, 6-chloro-2-methylphenol and 4-chloro-2-methylphenol were significant impurities in the herbicide 4-chloro-2-phenoxyacetic acid which the agrochemical plant happened to be producing.

Baigrie[10] describes an instance where an antiseptic type flavour was thought to be caused by a chlorophenol; however, some members of the tasting panel believed that the off-flavour did not have all the attributes of a true chlorophenol off-flavour – subsequent analysis showed that the compound responsible was *N,N*-dimethylaniline.

Elegant studies by Curtis *et al.*[11] showed that *Aspergillus* and *Penicillium* species can methylate chlorophenols to produce chloroanisoles and these were ingested by poultry which then exhibit intense off-flavours when

[10] B. D. Baigrie *Food Manuf.*, 1988, August, 21.

[11] R. F. Curtis, C. Dennis, J.M. Gee, N. M. Griffiths, D. G. Land, J. L. Peel, and D.G. Robinson, *J. Sci. Food Agric.*, 1974, **25**, 811.

cooked. A puzzling problem of a mouldy off-flavour in meat exported from Australia revealed that the problem was caused by condensate in chiller rooms flooding insulation material which contained a small amount of phenol. The chiller rooms were sterilized by means of chlorine, which reacted with the phenol, which was in turn methylated by fungus present in the building material. Forced air circulation then transported the compound throughout the building. The problem was solved by closing down the chiller complex and rebuilding.[12]

To indicate that the problems are still in existence today, there is much work on 'cork taints' in alcoholic drinks, *e.g.* Cognac[13] and wine.[14–16] Some taints are due to treatment of cork trees with fungicides and others are due to contamination of damp warehouses by fungi. Careful analysis is required to identify the true cause of the problem.

Other types of off-flavour can be caused by printing inks and paint solvents containing styrene and naphthalene. A particularly nasty taint can be caused by compounds which contain ester or ketonic functional groups such as methyl vinyl ketone.[17,18] The ketones react with sulphydryl groups in the food causing catty odours which are particularly noticeable and offensive. Outbreaks have occurred in a wide range of foods such as carrots (solvent residues from pesticides), pork luncheon meat (residual solvent in can lacquers), rice (printing ink on paper sacks), and cakes (airborne contamination produced by the interaction of ketones and sulphur compounds in a chemical factory 20 miles away from the bakery).[4]

Swoboda and Peers[19] have attributed the occurrence of cardboard flavours in foods to the presence of saturated or unsaturated aldehydes and metallic flavours to unsaturated ketones. These compounds can occur in foods by oxidative processes. It is interesting to note that some cardboards can contain up to 2% by weight of non-polar lipid – in other words, the 'cardboardy' description is derived from the fact that paper has undergone oxidation.

4 Case Studies

In recent years a wide range of off-flavours and taints have been identified in a variety of foods (Table 1). Some of these have involved extensive detective work to elucidate the various causes. Successes in identifying these causes have been the result of close collaboration between food

[12] CSIRO Division of Food Research, Report of Research 1981–82, p. 25.
[13] R. Cantagrel and J. P. Vidal, in 'Flavours and Off-flavours', ed. G. Charalambous, Elsevier, Amsterdam, 1990, p. 139.
[14] H. Tanner, C. Zanier, and G. Wurdig, *Schweiz. Z. Obst. Weinbau*, 1981, **117**, 752.
[15] H. Tanner, C. Zanier, and H. R. Buser, *Schweiz. Z. Obst. Weinbau*, 1981, **117**, 97.
[16] P. Dubois and J. Rigaud, *Vignes et Vins*, 1981, **301**, 48.
[17] T. J. P. Pearce, J. M. Peacock, F. Aylward, and D. R. Haisman, *Chem. Ind. (London)*, 1967, 1562.
[18] F. Aylward, G. Coleman, and D. R. Haisman, *Chem. Ind. (London)*, 1967, 1563.
[19] P. A. T. Swoboda and K. E. Peers, *J. Sci. Food Agric.*, 1977, **28**, 1019.

technologists, analytical chemists, and sensory panels. Others defied solution; only theories could be put forward.

Table 1 *Food tainting problems – some products and the causes*

Disinfectants
Tomatoes – fresh
Coated fish products
Tomato juice

Chlorophenols
Milk
Water
Cardboard packaging
Sugar solutions
Grain
Fish paste
Canned beans
Red kidney beans

Chloroanisoles
Strawberry jam
Cocoa butter

Solvents/lubricants
Coated fish products
Biscuits
Pineapple
Lamb (also contained naphthalene)
Rice
Corned beef

Sulphur taints
Cider
Wine

Bacterial/fungal
Orange juice – acetoin
Fried potatoes – ethyl butyrate
Frozen pastry – ethyl butyrate

'Catty' taints
Meat products

Enzyme reactions
Pizzas

Miscellaneous/unidentified sources
Milk – faeces odour – methylindole
Apples – naphthalene
Apples – blue–green discoloration plus a metallic flavour
Custard – cabbagy off-flavour
Corned beef – musty flavour
Ice-cream – rancid palm oil
Poultry – contamination from plastic trays
Wine – contamination by non-food grade plastic tube

The most common problems, in terms of numbers of outbreaks, have been due to chlorophenols and chloroanisoles. The means of contamination have sometimes been quite unusual – for example, greenhouse-grown fresh tomatoes absorbed chlorophenols from traces of these compounds brought into the house from a sanitizing footbath outside the entrance. A coated fish product was tainted by a disinfectant used on board a trawler – the catch by this boat was purchased by a processor and mixed with other lots. Eventually the whole day's production in a fish processing factory was contaminated.

Process water caused chlorophenol problems – the mains supply to a factory was changed, the new supply being abstracted from a different source and containing phenols derived from humus. A similar problem occurred in a pea cannery. These problems came to light when contaminated product was detected during quality control operations. Others were caused by residual cleaning materials following clean-in-place operations or by too high a level of free chlorine in water used for fluming.

Chloroanisoles were detected in cocoa butter. The origin of these was suspected to be fungal methylation of chlorophenols during fermentation of the cocoa beans in the source country. The reason why chlorophenols were

present in the first place is unknown as the field history of the crop could not be traced. In the case of the strawberry jam, chloroanisoles were detected in cardboard boxes used for transporting the fresh fruit.

Solvent or lubricant taints can usually be explained by accidental contamination during processing or storage, or by storage of food near to products releasing tainting compounds. Departures from good manufacturing practice are often the cause of this type of problem, for example where containers in a factory are used for non-food purposes. The tainting of frozen desserts was caused by solvent release from mastic used for sealing around holes for pipes running through a wall. This tainting process occurred at −40 °C in a freezer store.

Similarly, bacterial or fungal taints are usually attributable to abuse of the food. The fried potatoes in Table 1 had a cheesy–sour flavour caused by *Pseudomonas* spp. producing ethyl butyrate, as did the frozen pastry. The orange juice contained acetoin, either as a result of over-processing or bacterial contamination. The colour of the product favoured the over-processing theory and no significant number of dead bacterial cells were found. A problem which can occur in a warm pea season is that of 'vining flavour', where mechanically harvested peas are held for too long before entering the factory and being processed. This is a combination of chemical (mainly) and microbiological deterioration.

A most interesting problem presented itself with frozen pizzas, occuring spasmodically during several days production. The pizzas were garnished with fresh sliced capsicums and it was eventually established that, in some consignments of mozzarella cheese, a compound or compounds reacted with some component of the peppers to cause the effect. No effect could be produced with blanched peppers, and efforts to duplicate the reaction using other batches of cheese and peppers failed. The effect was assumed to be enzyme moderated because the intensity of the effect could be changed at different storage temperatures. In view of the limited time and quantity of material available, no analytical work was carried out but the basic cause of the off-flavour was established beyond reasonable doubt.

Several examples of off-flavour problems have proved to be insoluble. Why, for example, did frozen bramley apple turn blue–green on thawing and simultaneously develop a metallic off-flavour? What caused a custard product to taste of cabbage? Where did fresh apples become contaminated with high levels of naphthalene? In these cases the level of occurrence of the problem was very small, but by those who suffered each episode was considered to be very important because somewhere, something had gone wrong and for an ethical industry much effort had to be expended to characterize the problem in order to prevent its recurrence. In these problems it was not possible to find the root cause of the contamination.

5 Prevention

What can the food manufacturer do to reduce the possibility of tainting

chemicals entering the product? The number of problems encountered by the industry is growing despite the increased knowledge base that is being accumulated. There are more sources of chemicals being recognized as causing taint in food. Some possible reasons may be the use of new packaging materials and the current concerns about migration of components into the food, the increased expectations of the consumer, the increased use of water which may carry traces of industrial chemicals, problems of air and soil pollution, and finally, perhaps, the use of bulk transport containers which have been used to carry odoriferous materials.

To combat these, the food processor must check every aspect of the process and ensure that a good quality control system based on BS5750/ISO9000/EN29000 is implemented. Many companies in the food and allied industries are now doing this. In the case of possible air-borne contamination, food technologists should be aware of likely problems from nearby chemical factories; new food factories and warehouses should be sited well away from sources likely to produce contamination. In the case of packaging, careful attention should be paid to specifications and these should be checked regularly after the initial drawing-up. Water used for processing or as an ingredient should be monitored to ensure that pollution has not occurred. Paints and flooring materials should be allowed to cure before food processing takes place in a building which has been erected or refurbished. Process plant and buildings should be designed and maintained so that pockets of soil where bacteria and fungi may multiply are not present. Agricultural chemicals used on crops should be screened for their propensity to produce taints or off-flavours. Sub-contractors and suppliers should be advised of the problem of taint and warned to take precautions.

If all these points are taken into consideration, then the likelihood of a taint problem occurring will be lessened and the question 'Who is to blame?' will not be heard as often.

Subject Index