7 86

Information Section

THE LEON GOLBERG MEMORIAL LECTURE

BIBRA Toxicology International Annual Scientific Meeting, 3 February 1993

Dr Francis Roe
19 Marryat Road, Wimbledon Common, London

RECENT ADVANCES IN TOXICOLOGY RELEVANT TO CARCINOGENESIS: SEVEN CAMEOS

Cameo I: A battle of giants

I first met both Leon Golberg and Frank Fairweather in 1961, shortly after my appointment as Reader in Experimental Pathology at the Chester Beatty Research Institute. The meeting, which took place in the office of the Institute's Director, Professor Alexander Haddow, before he received the royal accolade, concerned the ease with which one could produce sarcomas in rats by injecting iron dextran (Imferon) into them subcutaneously. At that time, Leon Golberg and Frank Fairweather were on the staff of Benger's Laboratories, who marketed Imferon. The atmosphere in Haddow's office was tense. Leon pointed out that the rats in Richmond's (1959) and Haddow and Horning's (1960) experiments that developed sarcomas had received overwhelmingly large doses of iron on a mg/kg/body weight basis. Unmoved by this argument, Haddow emphasized that sarcoma induction was a local phenomenon and that a 0.5-ml subcutaneous dose of iron dextran in the rat came into contact with approximately the same number of connective tissue cells as a similar sized dose in a human. The discussions reached an impasse because neither argument could be refuted on the basis of available scientific evidence.

The position in 1961 was quite simply that any chemical that increased the incidence of any form of cancer irrespective of dose or route of administration was by definition a carcinogen and therefore a potential risk for man. Not only was this the generally accepted view in the early 1960s but there were those who thought that tests involving parenteral injection should be used for food and other chemicals that were poorly absorbed from the gut (Boyland, 1950; Gross, 1961). Persuaded by this view, the medical establishment recommended the speedy withdrawal of iron dextran from the market despite its acknowledged value in the treatment of iron deficiency anaemia (*British Medical Journal*, 1960).

Golberg's response to this was to embark on a programme of laboratory research aimed at showing why the findings in rats were not applicable to humans. However, another quarter of a century had to elapse before the concept of non-genotoxic carcinogenesis began to trip off the tongues of cancer researchers.

Baker *et al.* (1961), using radiolabelled iron dextran, found that retention of iron at the injection site was higher in the rat — particularly the male rat — than in the mouse, rabbit, dog or man; and Golberg *et al.* (1961) reported that the local response to injected iron dextran — in the form of necrosis, accumulation of iron-laden macrophages, active fibroblastic activity and severe fibrosis — was more marked in the rat than in other species. By comparison histological sections prepared from gluteal injection sites in patients given courses of injections of iron dextran up to 3 years previously showed no necrosis or fibroblastic activity. In the light of these and other data, it was postulated that, by reducing the dose introduced into any one injection site, necrosis, fibrosis and the risk of neoplasia could be avoided. This proved not to be entirely true in the rat. Thus, when we (Roe *et al.*, 1964) compared the response of rats given multiple injections of iron dextran into the same subcutaneous site with that in rats given the same number of injections but distributed between two, four or six different sites, we found that the total number of sarcomas that developed increased with the number of injection sites used, although the length of the latent interval between first injection and tumour development was inversely related to the number of injection sites.

It was also postulated that species in which iron was more efficiently removed from the injection site (e.g. rabbit and man) would be less susceptible than the rat to the induction of sarcomas. In practice, however, it proved easy to produce sarcomas in rabbits by giving them repeated intramuscular injections of iron dextran (Haddow *et al.*, 1964).

Early on it was shown that the dextran moiety of iron dextran (Haddow and Horning, 1960) did not give rise to injection site tumours, and it seemed unlikely that iron *per se* was carcinogenic insofar as no increased incidence of cancer was ever seen at the sites (e.g. the liver) to which the metal was carried from the injection site (Golberg and

Smith, 1960a). The most plausible hypothesis seemed to be that iron dextran is not carcinogenic *per se*, but if its injection leads to persistent necrosis, fibroplasia and fibrosis, there is an increased risk that these changes will progress to sarcoma development and, furthermore, that gross overloading of animals with iron increases and prolongs the state of necrosis, fibroplasia and fibrosis by slowing the rate of its transport away from the injection site. Golberg and his colleagues carried out numerous carefully documented studies that support this concept (Golberg *et al.*, 1960 and 1961; Golberg and Smith, 1960b; Muir and Golberg, 1961).

Up to 1977 eight possible cases of sarcoma arising in humans at the site of previous injection of iron had been reported (Greenberg, 1976; MacKinnon and Bancewiez, 1973; Robertson and Dick, 1977; Robinson *et al.*, 1960). In some cases the shortness of latent interval between injection and sarcoma development (e.g. just a few months) cast doubt on the causal nature of the association. In other cases, the impossibility of confirming the identity of what had been injected many years previously and the absence of stainable iron in the vicinity of the sarcoma weakened the indictment of iron-injection as the cause. An investigation of 90 cases of sarcoma of the buttock revealed that in only four was there a history of intramuscular injections of iron (Greenberg, 1976). Unfortunately, as pointed out by Metcalfe (1976), the lack of information about the incidence of previous intragluteal iron injections in a comparable control group without sarcoma rendered the results of this investigation uninterpretable.

In my view the battle of giants ended as a draw. Whether the unnecessary use of parenteral iron preparations led to the occurrence of a single case of injection-site sarcoma in a human is very doubtful. However, it is undoubtedly true that 30 years ago many people, particularly pregnant women, were being given parenteral iron injections which they did not need (Haddow, 1960). Some of the physicians involved simply overlooked the fact that normal pregnancy is associated with haemodilution and with levels of haemoglobin that are 10–15% lower than those associated with the non-pregnant state. Furthermore, despite the fact that it has been shown that parenteral injection of iron is only of value if bone marrow iron stores are low (Davies, 1971) not all of the syringe-happy physicians took the trouble to establish that iron deficiency was the cause of pathologically low haemoglobin levels. The contribution of Golberg and his colleagues was that they brought logic and careful laboratory research to bear on the problem and began to lay the foundations of our present-day understanding of non-genotoxic mechanisms involved in carcinogenesis.

Cameo II: Does it get under your skin?

Boyland (1958) wrote "Food additives should be tested both by injection and by feeding, but the injection test is the more stringent and desirable". When he wrote this, he was not out of tune with the thinking of the time. How-ever, Leon Golberg was sure that this view was wrong, and one of the first programmes of research that he launched after his appointment as the first Director of BIBRA in 1961 was to assess the value of subcutaneous sarcoma induction as an index of carcinogenic activity. During the years that followed there appeared a series of papers by Paul Grasso, Leon Golberg, Sharat Gangolli and Jean Hooson that steadily eroded faith in the appropriateness of the subcutaneous injection method for testing substances for carcinogenicity, particularly substances for which exposure was normally by the oral route.

Grasso and Golberg (1966b) reviewed many different aspects of this problem. They distinguished between 'true' carcinogens, 'weak' carcinogens and substances that act only non-specifically and indirectly to produce sarcomas. 'True' carcinogens were further subdivided into (i) those that produce sarcomas, and maybe other tumours, at the site of injection **plus** tumours at sites distant from the injection site, and (ii) those that only produce tumours at distant sites. They considered the influences of the dose administered, the solvents used and the relationship between the production of sarcomas by the injection of chemically unreactive compounds and their production by implants of metal, glass, plastic and other solid objects. They commented on the fact that certain undoubted carcinogens had not been found to produce sarcomas at the site of parenteral injection. However, they were working before the importance of metabolic activation of procarcinogens to carcinogens by endogenous enzymes was understood. Their tentative conclusion was that physicochemically-mediated trauma leading to derangement of connective tissue repair can lead non-specifically to the development of sarcomas. In support of this tentative conclusion they were able to refer to their findings in studies of changes at the site of repeated injections of certain food colourings (Grasso and Golberg, 1966a) and, of course, the results of studies on iron dextran (*vide supra*). Gangolli *et al.* (1967) showed that the nature and extent of local tissue reaction was not closely related to retention of the injected material at the injection site. More important determinants of local tissue response were surface activity, lipid solubility and protein-binding activity, particularly if it led to alteration of protein structure. Hooson and Grasso (1970) found that early tissue response to water-soluble true carcinogens was quite different from that to those materials that produce injection-site sarcomas. Instead of the marked and prolonged fibroblastic activity associated with the latter, there can occur an inhibition of fibroblastic proliferation and of granulation tissue formation. Grasso *et al.* (1971) showed how calcium ion concentration and modification of the surface activity of the material injected could determine both early local tissue response and risk of subsequent sarcoma development.

In the light of this carefully conducted programme of research, it would nowadays be difficult to find a respected toxicologist who would recommend screening food constituents, additives or contaminants for carcinogenicity using parenteral injection as the route of administration.
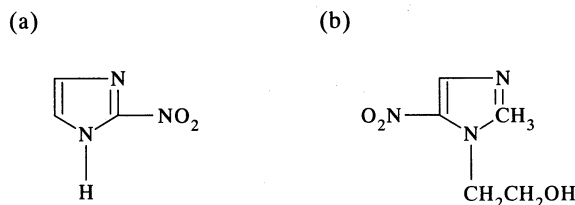
(a)   (b)

Fig. 1. (a) Azomycin derived from Streptomyces sp: 2-nitroimidazole and
(b) metronidazole (Flagyl): 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole.

Nevertheless, there are, unbelievably, still some who defend the use of intrapleural or intraperitoneal injection as a suitable method for evaluating possible carcinogenic risk from inhaled fibres and other particles (Pott *et al.*, 1987). In the light of the research on subcutaneous injection carcinogenesis, it is my strongly held view that no reliance should be put on the results of injecting dusts or fibres into serosal spaces.

Cameo III: Beware of occlusive knickers

Trichomonas vaginalis is a venereal disease. Describing the situation in the 1950s, Duncan Catterall (1977) wrote:

"At the majority of clinics there were queues of sad-looking, unhappy women . . . with wet and stained underwear and poor morale. Nor was there any satisfactory treatment to offer them."

Towards the end of 1954 Rhône-Poulenc began to look for a drug that would be effective against the causative parasite and thereby offer relief to the millions of women throughout the world who were infected with it. A step forward was made when Despois *et al.* (1956) found that a crude extract of the strain of Streptomyces was active against the parasite. The active principle was azomycin (Fig. 1). Numerous analogues were synthesized and tested and 4 years later Cosar and Julou (1959) reported the successful treatment of mice infected with *T. vaginalis* by the oral administration of metronidazole (Fig. 1). Soon after this, the drug was found to be effective clinically (Durel *et al.*, 1959) and was christened 'Flagyl'.

Since 1960, trichomoniasis has been a treatable disease. Local intravaginal use favoured initially, particularly in France, soon gave way to oral treatment, which proved more effective. Courses of treatment lasting over 7–10 days gave way to a single large-dose treatment (e.g. 2 g per person). The need to treat sexual consorts at the same time as patients themselves became widely recognized. Fortunately, drug resistance never became a problem, probably because there was no margin between the toxic concentration of metronidazole and the concentration that was lethal for the protozoon. Other nitroimidazoles were developed and marketed for the treatment of the

disease but none proved more effective than metronidazole itself.

This might have been the end of the story had it not been for the chance observation of David Shinn, a dental surgeon at King's College Dental School in London, and for the wisdom and dogged persistence of Dr J.A. McFadzean, the Research Director of the May & Baker subsidiary of Rhône-Poulenc. Shinn, who had for some time with only limited success been treating a woman for Vincent's angina, a painful and distressing infectious disease of the oral cavity, was surprised when one day it suddenly cleared up. On enquiring, he found that the lady in question had also had problems at the other end of her body in the form of trichomoniasis. A course of Flagyl had been equally effective for her mouth and for her vagina. Shinn contacted McFadzean and a pilot study that confirmed the efficacy of metronidazole in the treatment of Vincent's disease was reported (Shinn, 1962).

The question then was: why did Vincent's angina respond to the drug? The disease had been reported to be associated with the presence in the mouth of large numbers of spirochaetes and fusiform bacilli, and although treatment with metronidazole led to the disappearance of these organisms from the mouth, another important development was necessary before the true cause of the disease and the reason for its response to metronidazole could be understood.

In the late 1940s the chances of a patient who had bowel surgery—even a simple appendicectomy—or a hysterectomy developing a post-operative wound infection ranged up to 50%. Swabs taken from many of the wounds were reported to be 'sterile'. Nevertheless, wounds continued to produce pus and were very slow to heal.

Table 1. Results of prophylaxis trial of metronidazole in patients undergoing hysterectomy

Treatment	No. of patients	Anaerobic sepsis rate (%)
Metronidazole umbrella	74	1
No metronidazole	60	30

Study Group Report (1975)

The breakthrough in understanding this situation came with two discoveries. First a report appeared (Drasar *et al.*, 1966) indicating that 98% of the bacteria present in the large intestine are anaerobic in type and belong to the Bacterioides group, whereas less than 0.1% are gram-negative bacilli (e.g. *Escherichia coli*). However, the recognition that many wound infections that produce apparently sterile pus are in reality due to obligate anaerobic bacteria had to await the development in the early 1970s of laboratory methods for growing these bacteria from swabs taken from patients. As soon as such methods became available, it became apparent that metronidazole was highly active in killing these bacteria (Nastro and Finegold, 1972).

In 1974 Trevor Willis, Director of the Public Health Laboratory in Luton and a leading authority on anaerobic bacteria, managed to persuade his clinical colleagues at the Luton and Dunstable Hospital to carry out a prophylactic trial of metronidazole as an 'umbrella' during hysterectomy (Study Group, 1974). As shown in Table 1, the results were dramatic (Report of Study Group 1975). Unfortunately, however, the medical establishment, which claims to be always on the look-out for breakthroughs, sometimes fails to recognize them when they happen. The uphill struggle against scepticism that Willis and his colleagues faced is graphically described by McFadzean (1986), whose dogged determination to find a new use for an old drug met with comparable resistance within Rhône-Poulenc, and led to his being dubbed 'Dr McFlagyl'.

Nowadays, as is well known, metronidazole is more or less routinely used as a prophylactic against post-operative infections by anaerobic bacteria in the course of bowel and gynaecological surgery. It also has a place in the treatment of numerous other infectious conditions now recognized as being due to obligate anaerobic organisms (Finegold, 1977; Willis, 1977). However, before this happy state of affairs emerged one further hurdle had to be cleared.

Long-term tests in rodents indicated that prolonged exposure to very high doses of metronidazole increased the risk of lung tumours and malignant lymphoma development in mice (Rust, 1977; Rustia and Shubik, 1972) and that heavy exposure of rats increased the incidence of a variety of neoplasms (Rustia and Shubik, 1979). Furthermore, positive results were reported in some tests for genotoxicity (Mitelman *et al.*, 1976; Rosenkranz, 1977; Voogd *et al.*, 1974).

The mutagenic activity of metronidazole is attributable to the nitroreductase activity possessed by the obligate anaerobic bacteria that it kills. Aerobic organisms and healthy mammalian cells do not possess such activity. The laboratory evidence of genotoxicity was, therefore, not relevant to man. A remarkable aspect of virtually all the long-term tests in rats, mice and hamsters was that exposure to metronidazole led to improved survival compared with controls. Overnutrition is associated with markedly increased incidences of lung tumours and malignant lymphoma in mice (Conybeare, 1980; Roe and Tucker, 1974). It is probable that, like other antimicrobial

agents that affect the gut flora, metronidazole improves the nutritional status of mice and consequently has a similar effect to overfeeding (Roe, 1977). The failure of Rustia and Shubik (1979) to distinguish between fatal and incidental tumours and to age-standardize the data they obtained in their rat study rendered their findings uninterpretable.

Such data as have been published are consistent with there being no increased risk of cancer in humans after exposure to metronidazole. Beard *et al.* (1979) followed up 771 women who received metronidazole for trichomoniasis during the period 1960 to 1969. A comparable group of 237 sufferers from the disease who received no metronidazole were available for comparison. In both groups the incidence of carcinoma of the cervix was higher than that for the general population. This was only to be expected in view of the well known association between the risk of this form of cancer and what is euphemistically referred to as 'poor sexual hygiene'. Apart from this the only excess of cancer risk seen in the metronidazole treated group was four cases of lung cancer instead of only 0.6 cases expected. The four women in question were smokers. A follow-up under the Kaiser-Permanente programme of nearly 2500 women given metronidazole between July 1960 and August 1973 revealed no evidence of any excess of cancer at any site (Friedman and Ury, 1980).

Some time in the early 1980s I found myself attending a meeting of a US-FDA Committee concerned with the safety of using metronidazole in the treatment of trichomoniasis. To my amazement and horror a lady sitting next to me told those assembled that in her view there was no need to treat women with a proven carcinogen. She herself had suffered continuously from trichomoniasis for about 20 years but had found that she could easily live with it provided that she had a hot bath each day and avoided wearing occlusive knickers! I vividly remember the deafening noise of chairs being edged away from where she was sitting.

Happily today metronidazole is widely used both in the treatment of trichomoniasis and other protozoal diseases and in the prevention and treatment of numerous conditions caused by anaerobic bacteria, and the benefits in terms of reduced suffering and the average length of stay in hospital are enormous.

Cameo IV: Lumps and bumping off

Early in the 1970s I asked Richard Peto to help me to interpret the results of a long-term skin painting study in mice. Some of the treatments were associated with the development of malignant tumours of the skin and for humane reasons it was necessary to kill these animals. In consequence, there was an inverse relationship between survival and skin tumour incidence. But I wanted to know whether the different treatments to the skin were associated with different risks of development of lung tumours. Peto's first approach was to conduct an actuarial analysis

Table 2. Data relevant to carcinogenesis available from
laboratory animals and man

	Laboratory animals	Man
Autopsy rate (%)	100	< 20
Thoroughness of autopsy	High	Variable
Reliability of data		
Tumour incidence	High	Very poor
Cancer mortality	Poor	Moderate
Influence of therapy	–	High

which assumed that mice found at autopsy to have lung tumours died because of these tumours. I explained to him that most lung tumours in mice are benign and very slow growing. Thus, when a small lung tumour is found at autopsy in a mouse dying or killed because it has a skin tumour or for some other reason, there is no justification for regarding the lung tumour as in any way contributing to the animal's death. Such an animal might well live for many months longer with its lung tumour growing slowly but not impairing its health and then die from some

unrelated cause. Richard considered what I had said and questioned me about the likely contribution to death of various other internal tumours (such as hepatomas, interstital cell tumours of the testis, C-cell tumours of the thyroid, benign phaeochromocytomas etc.) that are found in long-term rodent studies. I told him that in the vast majority of cases such lesions are simply incidental findings. After further consideration he concluded that this meant that the traditional way in which the results of carcinogenicity studies in animals had always been analysed had been entirely wrong.

Armed with this new insight and his own boundless energy Richard set about rectifying the matter, first with a short leading article in the *British Journal of Cancer* (Peto, 1974) and later, together with a quorum of other statisticians, with an unduly long Supplement to the IARC Monograph Series (Peto *et al.*, 1980).

Nowadays, most experimentalists understand the need to try to distinguish between fatal and incidental lesions, and reports on carcinogenicity studies include lists of lesions considered to have contributed to premature death in laboratory animals.

However, the situation is still far from satisfactory. Some animals die for no apparent reason. Others are so

Table 3. Percentage of deaths in1991 for which autopsy was performed (WHO, 1992)

Country	All deaths	Deaths in different age groups		
		15–44	45–64	65+
Hungary	49	?	?	?
Sweden	37	76	56	36
England and Wales	26	62	36	22
Scotland	15	57	24	11
USA	12	15	16	5
West Germany	8	?	?	?
Netherlands	8	14	11	7
Japan	4	8	7	3

Table 4. Examples of false negative and false positive rates (%) revealed by autopsy in the case of primary cancer of the lung

Country	Year	Clinical diagnosis		Cancer only seen at autopsy	False -ve	False +ve	Ref.
		Correct	Wrong				
England and Wales	1966	227	111	190	45.6	32.8	(1)
Japan	1967	1488	305*	788	34.6	17.0*	(2)
Australia	1967	43	9	28	39.4	17.3	(3)
USA	1971	27	34	0	0	55.7	(4)
USA	1980	260	38	117	31.0	12.8	(5)
Scotland	1981	88	15	46	34.4	14.6	(6)
Italy	1986	72	27	62	46.3	27.3	(7)

*Total includes only cases diagnosed as cancer of other sites.
References: (1) Heasman and Lipworth (1966); (2) Takeda and Kobayashi (1967); (3) Willis (1967); (4) Rosenblatt *et al.* (1971); (5) Cechner *et al.* (1980); (6) Cameron and McGoogan (1981 a,b); (7) Mollo *et al.* (1986).

full of potentially 'fatal' lesions that it is difficult to know how they clung to life for so long and appeared so well. Faced with such difficulties, it is all too common practice for computers to make the decisions about which lesions contributed to death. Thus, the computer may be programmed to list any malignant neoplasm, irrespective of its site or size, as such a factor. Where this happens, small well differentiated doubtfully malignant lesions that happen to lie in the path of the microtome knife are bracketed together with massive lesions described in graphic terms by prosectors.

I doubt whether it will ever be possible to produce accurate cancer mortality data for laboratory rodents. However, there is certainly scope for improving the way in which things are done at present.

In any case, there is, and will remain, a serious gap between the kind of data produced from carcinogenicity studies in rodents and the kind of data available to epidemiologists concerned with cancer incidence in man (see Table 2). For most kinds of cancer arising internally the only data that are available for humans are mortality data. Currently, very low autopsy rates (Table 3) (e.g. only 5% in the USA and only 3% in Japan for persons over 65), combined with serious discrepancies between clinical and autopsy diagnoses (e.g. Abrahamson *et al.*, 1971; Alderson *et al.*, 1983; Heasman and Lipworth, 1966; Lee, 1993; Rosenblatt *et al.*, 1971; Royal Colleges Report, 1991; Takeda and Kobayashi, 1967; Willis, 1967) and also discrepancies between findings at autopsy and entries on death certificates (James *et al.*, 1955; see Lee, 1993 for review; Steer and Land, 1976) render even these data extremely inaccurate. As an illustration of how unreliable human cancer mortality data are, the results of seven studies on lung cancer are summarized in Table 4.

Thus, the position is that for small non-fatal neoplasms arising in internal organs there is no reliable source of data for humans. By contrast, for laboratory animals we have an abundance of data derived from detailed and more or less standardized autopsy procedures on the incidence of benign and malignant neoplasms but, for the reasons discussed above, only rather unreliable cancer mortality data.

On the accuracy of the post-mortem diagnosis Wilson (1966) wrote ". . . in justice and honesty it must be admitted that in a certain number of patients—about 20%—more or less mystery and confusion still prevailed after post-mortem examination". Despite my claim to be a pathologist, I accept that the autopsy room is not the 'palace of truth' it has been claimed to be by some pathologists and feared to be by some clinicians. It would be less misleading to think of it as a place where one can come closest to the truth about the cause of death.

In conclusion, it seems to me a serious matter that the importance of the distinction between tumour incidence data derived from animal tests and human cancer mortality data is largely unknown to, and unappreciated by, activists, medical science writers, and many of those who sit on regulatory bodies and who advise politicians.

Cameo V: Danger in the air: beware of consensus

I was born and reared in the sulfurous fog of south-east London long before the Clean Air Act. Staying indoors provided me with little protection from air pollutants because only one room in our house was heated. In this small living room my father continuously smoked his pipe, the open fire intermittently delivered its fumes into the room instead of up the chimney, and my grandmother, having unilaterally declared south-east London to be a moth-free zone, sat in a haze of camphorous fumes. I look back in wonder that I both tolerated and survived all these insults to my lungs. By contrast, I am today thoroughly intolerant of intrusive odours and particularly of irritation from other people's tobacco smoke.

In the early 1950s, laboratory studies in the field of tobacco carcinogenesis were confined to applying tobacco smoke condensate repeatedly to the dorsal skin of mice. In the early days negative results were often obtained either because of the Scylla of insufficient dosage, or because of the Charybdis of excessive dosage, which led to nicotine poisoning. Even if these hazards were successfully circumnavigated there remained a high risk of premature death from intercurrent disease. Eventual success in producing skin tumours with whole smoke condensates (Wynder *et al.*, 1953) led on to skin painting experiments using fractions and sub-fractions of the condensate in the hope of pinpointing the carcinogen or carcinogens responsible for both the skin tumours in mice and the lung cancers in smokers. A sub-fraction of the neutral fraction that contained, *inter alia*, the polycyclic aromatic hydrocarbons (PAH) and related compounds proved to be most active (Whitehead, 1977). However, irritants in other fractions of the condensate, such as the phenolic fraction, had previously been shown to exhibit a tumour-enhancing effect (Roe *et al.*, 1959). In the case of the PAH in the neutral fraction, attention was focused on the known carcinogen, 3,4-benzopyrene (BP), because its characteristic UV absorption spectrum rendered it easier to measure than other constituents of this fraction. However, we soon showed that this particular carcinogen contributes little to the carcinogenicity of smoke condensate for mouse skin (Roe, 1962). The tobacco industry spent huge amounts of money on such research, hoping, of course, that a single culprit carcinogen would be identified and that by a bit of clever manipulation with the raw tobacco or with the smoking process non-carcinogenic cigarettes could be produced.

Unfortunately, no single constituent of smoke condensate that could explain all its carcinogenic activity for mouse skin was found. Consequently, attention was switched to reducing the amount of tar delivered by cigarettes and to developing tobacco substitutes that, on pyrolysis, produced a simpler smoke with a lower PAH content. The first generation of products containing tobacco substitutes, which, incidentally, were given no tax advantage by Government, failed to satisfy smokers. Because of this and because of vigorous and, in my opinion, misguided opposition by anti-smoking cam-

paigners, no improved second generation products that might have been more acceptable to smokers were developed. More recently, there has been the same misguided opposition to the novel nicotine-delivery system called **Premier**, developed by the Reynolds Company, which probably carries no cancer risk at all for smokers (Reynold, 1988). This unhelpful opposition will certainly ensure that smokers will remain at unnecessarily high lung cancer risk well into the next century, it being wholly unrealistic to imagine that tobacco smoking can be eliminated merely by a combination of propaganda and heavy taxation.

Be that as it may, even in the 1950s and 1960s there were those who felt that mouse skin painting was not really appropriate or adequate for investigating a potential inhalation hazard. The smoke condensate applied to the dorsal skin of mice was not really fresh and, therefore, did not contain reactive chemicals with very short half-lives. Secondly, the mice were not exposed to the several irritants present in the vapour phase of smoke. And, thirdly, the epithelium of the lung is quite different from that of mouse skin. Early attempts to overcome some of these objections involved the intratracheal instillation of tobacco smoke condensates directly into the lungs of rats—sometimes alone and sometimes mixed with putatively chemically inert particulate matter such as carbon black particles. The addition of the particulate matter stemmed from the belief that the residence of inhaled carcinogens in the lungs might be prolonged by the presence of particles (Shabad, 1962). In my view, these experiments were really very crude. The distribution of the instilled material within the lungs was very much a matter of chance and the whole procedure bore very little relationship to exposure by inhalation during many hours per day, over periods of months or years. In any case, it now seems that the presence of particulate matter, such as carbon black, does not necessarily enhance carcinogenesis (Feron *et al.*, 1973; Henry *et al.*, 1973).

There were two possible approaches to exposing laboratory animals to tobacco smoke by the inhalation route. One was to confine them within a chamber into which fresh smoke was introduced, either continuously or intermittently. The other was to enclose each animal separately within a tube with its nose protrding at one end and to fix that end of the tube to a cylinder through which fresh smoke was passed continuously or intermittently. The latter method had the advantages of avoiding the absorption of smoke constituents through the intact skin and of ensuring that the smoke to which animals were exposed was fresh, but it had the great disadvantage of being extremely labour intensive.

With both types of approach nicotine poisoning and carbon monoxide poisoning were both potentially serious problems. The human smoker can interrupt exposure whenever he/she feels a bit queasy but a rat in a chamber or tube cannot do so. The only solution was to cut down the rate of dosing and/or to arrange for smoke exposure to be intermittent. A further insuperable problem was that by either method of exposure, all the smoke taken into the lungs of animals passed first through the fairly efficient filtration system provided by the nose, whereas most of the smoke that humans take into their lungs enters through the mouth. It was seemingly impossible to expose the lungs of rats and mice to as much smoke as a dedicated smoker can get into his/her own lungs. In one rat experiment (Davis *et al.*, 1975) the most prominent effect of exposure to smoke was a reduction in the incidence of mammary tumours (Table 5). Such effects on the lungs that were seen—including increased incidences of pigment-laden macrophages, cuboidal/columnar metaplasia of alveolar epithelium and squamous metaplasia of alveolar epithelium—were of doubtful relevance to the induction of lung cancers of the types associated with smoking in humans.

Looking back now, it is my view that laboratory research involving the exposure of animals to smoke by way of the inhalation route provided very few useful insights into the mysteries of tobacco carcinogenesis. However, some benefit certainly did stem from these experiments. New methods for exposing animals to chemicals by way of the inhalation route, for inhalation dosimetry and for the pathological assessment of the effects of exposure to industrial chemicals, fumes and dusts were developed—very often by those who had cut their teeth on tobacco smoke inhalation studies. For such chemicals, standard techniques are now available and widely used.

Table 5. Effect of inhalation exposure of female rats to cigarette smoke (10 cigs/wk)

Exposure	No. alive at wk			Total lung tumours	Total mammary tumours
	0	80	120		
Smoke	408	143	61	4	37
Sham	102	91	35	0[NS]	40[++]

NS = not significant   ++ = $P < 0.01$

from Davis *et al.* (1975).

Their relevance to environmental air pollution problems, on the other hand, is more dubious. The standard experimental protocol involves exposing animals to a test material for 6 hours per day on 5 days per week, excluding public holidays. Exposure to outdoor and indoor air pollutants, however, may extend through 24 hours per day on 7 days per week — as sometimes did my exposure

Table 6. Carcinogenicity of particulate air pollutants (AP) and/or cigarette smoke condensate (CSC) for mouse skin

Dose (mg) x 3 weekly to skin in acetone (0.25 ml)		Week when	
		25% of mice had skin tumours	10% of mice had malignant skin tumours
AP	CSC		
2	0	84	78
0	40	84	85
2	40	56	57

from Roe and Kearns (1967).

to London smog and camphor when I was a child. For many obvious reasons, one needs to be very careful in extrapolating from short-term high level exposure to long-term low level exposure.

Despite all the research that has been carried out, the overall position with regard to tobacco smoke is that we do not know which, if any, of the animal carcinogens that have been identified in smoke, is important. It is not certain that the PAH in the particulate phase are really important. Nor, despite much research of high quality (Hoffmann, 1989) is the role of tobacco-specific nitrosamines really clear.

25 years ago we (Roe and Kearns, 1967) reported that an acetone extract of particulate air pollutants collected by a sampler on the roof of St Bartholomew's Hospital was some 15–20 times more carcinogenic, weight for weight, than cigarette smoke condensate (Table 6). Three times weekly application of 0.25 ml of the acetone extractable material from 2 mg of particulate material collected on a Cambridge filter readily induced benign and malignant skin tumours in mice.

There are many members of the medical profession and of the general public who are so concerned with the strong association between cigarette smoking and lung cancer, that they regard it as justifiable to use any means to stop people from smoking. Among the means presently used is to make statements about the numbers of lung cancer deaths attributable to exposure to environmental tobacco smoke. From a logical viewpoint such calculations are unconvincing, if not ludicrous. Epidemiology is a blunt tool (Bailar, 1989) and one that is clearly too blunt to measure a possible small difference in lung cancer between non-smokers married to smokers as compared with non-smokers married to non-smokers. In such groups,

one cannot titrate risk against actual measurements of exposure to environmental tobacco smoke over a lifetime since no data for exposure exist, and in none of the 29 analysable studies of this kind (see Lee, 1992) was it possible to control reliably for all the other factors known to be important as determinants of lung cancer risk, such as radon (Clarke and Southwood, 1989), bird-keeping (Holst *et al.*, 1988; Kohlmeier *et al.*, 1992), outdoor air pollution (Roe and Walters, 1965), indoor air pollution (Chen *et al.*, 1990), genetic factors, occupational factors and diet (Koo *et al.*, 1988). People's statements about their own present and past smoking habits are extremely unreliable (Lee, 1988). And furthermore, mortality data for lung cancer are grossly unreliable (Table 4).

All we know for certain is that environmental tobacco smoke can be irritant to the nose and eyes and aggravate the symptoms of asthma. Whether these short-term effects have more serious long-term consequences has not been established. Unfortunately, the toxicologist has not been able, so far, either to support or refute the claims and counter-claims made in relation to lung cancer risk from environmental tobacco smoke, and decisions concerning the existence and extent of hazards are being made by what is billed as consensus (e.g. EPA, 1992). Some of the biggest mistakes in history have been based on consensus thinking. As pointed out by Skrabanek (1990), consensus should never be regarded as a substitute for scientifically based facts.

Cameo VI: On the road to Damascus

"And as he journeyed, he came near Damascus: and suddenly there shined round about him a light from heaven"
(*Acts*, 9 verse 3)

The development by Bruce Ames (Ames, 1971; Ames *et al.*, 1973) of a quick, cheap and sensitive test system for genotoxicity based on *Salmonella typhimurium*, had an electrifying effect on cancer research relevant to the detection of environmental carcinogens. First came the creed and then unrelenting effort to exploit it. The first version of the creed was "All carcinogens are mutagens". But this was quickly replaced in many people's minds by "All mutagens are carcinogens". The discovery that inert chemicals can, by what was called metabolic activation, be converted into mutagens provided a spur to unremitting efforts to show that any chemical that predisposes to cancer is in fact a mutagen or can be converted into a mutagen. Undeterred by negative results in 20 or more tests for genotoxicity, the ardent believer in the new faith would simply press on developing new tests for genotoxicity, being certain in his/her own mind that sooner or later the rogue chemical would be shown to be a mutagen.

The faith and the creed led to all sorts of useful substances coming under suspicion of carcinogenicity. It also led to the emergence of the 'one-molecule men', that

is to say of a clique of individuals who were prepared to argue that the exposure of the DNA of one body cell to one molecule of a mutagen could be enough to cause a cancer to arise.

As a pathologist who was seeing cancer in high incidence in untreated control animals as well as in animals deliberately exposed to potent genotoxic carcinogens, and as an experimentalist who was observing increased incidences of liver tumours in mice fed on diets containing increased levels of ordinary food ingredients such as groundnut oil (Gellatly, 1975) or sucrose (Hunter *et al.*, 1978) I was never tempted to chant these creeds. And later when I became aware of the increased incidences of adrenal medullary tumours and testicular (interstitial-cell) tumours in rats fed on a high lactose diet (Sinkledam *et al.*, 1983; Roe and Baer, 1985), I became convinced that genotoxicity was not a *sine qua non* of carcinogenicity. However, for a long time I had no insight into any alternative explanation.

The induction of malignant cancers by natural hormones—for example the induction of pituitary and ovarian cancers by removing the ovaries from an animal from their natural site and then implanting one of them back into the spleen (Biskind and Biskind, 1944)—was a clear example that naturally occurring substances as distinct from xenobiotic chemicals were capable of causing cancers to develop.

It was only when I read the paper by Bruce Ames (1983) that the picture began to become clear. His sudden recognition that all sorts of naturally occurring plant and other materials were genotoxic was seemingly a St Paul-on-the-road-to-Damascus-like revelation. But it was the other contents of that paper and of the later paper by Ames and Saul (1987)—particularly the fact that genotoxins are formed endogenously during the conversion of foodstuffs to energy — that really opened my eyes to the light. At long last I could see possible explanations of how natural hormones might cause cancer and of how dietary restric-

tion protected against it. From that time on I could see how non-genotoxic substances might indirectly give rise to genotoxic damage and to the cell-structure damage associated with ageing. 'Non-genotoxic carcinogenesis' became an acceptable concept and what I believe to be was the first international conference on "Nongenotoxic Mechanisms in Carcinogenesis" was held in Cold Spring Harbor in 1987 (Cold Spring Harbor Laboratory, 1987).

Cameo VII: Think before you eat

During the 1960s whilst I was at the Chester Beatty Research Institute I ran head-on into the problem of high tumour incidence in untreated control groups of animals. In mice the commonest, so-called 'spontaneous' tumours were of the lung, liver and lymphoreticular system. In rats, mammary, pituitary and other endocrine tissues were the most commonly affected. Unfortunately, the sites most frequently involved in spontaneous tumour development were exactly the same as those that constituted the endpoints in carcinogenicity studies. Irrespective of statistical significance, I was simply not persuaded that enhancement of tumour incidence from say 30% to 60% by a certain age could be taken as reliable evidence of carcinogenicity. Genetic factors were clearly implicated insofar as there were big differences between strains in spontaneous tumour incidence at particular sites. However, no strain of either rats or mice was immune to the problem in studies of 2 years' duration or longer. This situation, combined with the fact that the induction of sarcomas at the site of subcutaneous injection or implantation of virtually any chemical material into rodents was being accepted as proof of carcinogenicity was in my view making something of a nonsense of carcinogenicity testing.

My despair was only relieved when early in the 1970s I heard Mary Tucker present her data about the dramatic

Table 7. Some effects of restricting calorie intake of 80% of *ad lib.* in 1200-rat Biosure study

	Males	Females
Mature body weight	Down 20%	Down 20%
Survival to age of 133 wk	Up from 41% to 69%	Up from 34% to 76%
Reduction in non-neoplastic ageing-associated disease*		
Polyarteritis		---
Chronic myocarditis	---	---
Prostatitis	-	
Reduction in neoplastic disease (benign and/or malignant)*		
Any site	---	---
Epidermis and/or adnexa	-	-
Subcutaneous	-	-
Mammary	(-)	---
Pituitary – anterior lobe	---	---
Pituitary – intermediate lobe	--	--
Pancreas – islet-cell	--	
Pancreas – exocrine	-	
Lung	-	-

* = Age-standardized incidence: (-) = $P < 0.1$; - = $P < 0.05$; - - = $P < 0.01$; - - - - = $P < 0.001$.

Table 8. Effect of *ad lib.* feeding compared with restriction to 80% of *ad lib.* on relative risk of development of fatal or potentially fatal malignant neoplasms

Relative risk (95% confidence limits)	Males	Females
Of premature death	2.40 (1.61–3.59)	3.60 (2.42–5.59)
Of developing fatal or potentially fatal malignant neoplasms	4.80 (2.73–8.74)	3.34 (1.97–5.66)

(see Roe and Lee, 1991)

Table 9. Relative risks (RR) of (a) dying prematurely and (b) developing one or more benign or malignant neoplasms before the age of 133 wk in relation to body weight at the age of 29 wk

		Body weight quintiles at 29 wk					
	Sex	Very low	Low	Medium	High	Very high	Trend
No. of rats	M	120	120	120	120	118	
	F	120	121	118	122	116	
Relative risks of Premature death	M	1.00	1.23	1.56	1.85	2.56	$P < 0.0001$
	F	1.00	1.49	1.36	1.97	2.11	$P < 0.0001$
Benign or malignant tumour at any site	M	1.00	1.69	2.43	2.78	3.22	$P < 0.0001$
	F	1.00	1.39	1.33	1.96	1.69	$P < 0.01$
Malignant tumour at any site	M & F	1.00	1.36	1.76	2.71	2.93	$P < 0.0001$

M = male   F = female

reductions in spontaneous tumour incidence that could be brought about by simple dietary restriction. I persuaded her to join me in a joint presentation about the deficiencies in toxicity testing (Roe and Tucker, 1974) and ever since then have regarded it as a mission in life to convey the essential message that the *ad lib.* feeding of laboratory rodents with unnecessarily highly nutritious diets constitutes **overfeeding**, which predisposes to premature ageing and death, and to the development of a wide variety of benign and malignant neoplasms. The literature describing all this is abundant, going back to 1935 (Berg and Simms, 1960; Conybeare, 1980 and 1988; Deerberg *et al.*, 1990; McCay *et al.*, 1935; Masoro, 1984; Masoro *et al.*, 1989; Nolen, 1972; Ross and Bras, 1965; Ross *et al.*, 1983; Simms and Berg, 1962; Tannenbaum, 1940 and 1942; Tucker, 1979; Weindruch and Walford, 1988; Yu *et al.*, 1982) but I feel no shame in adding to it further (Roe, 1981 and 1991; Roe and Lee, 1991, Roe *et al.*, 1991; Salmon *et al.*, 1990; Turnbull *et al.*, 1985).

I believe that, at long last, heed is beginning to be taken of the situation, but there are still many who find reasons for not wanting to face the facts. Among the reasons is a belief that tumours can only arise because there has been exposure to a xenobiotic carcinogenic agent and that high spontaneous tumour incidence is merely a marker of high susceptibility to xenobiotic carcinogens. However, many naturally occurring chemicals are genotoxic and potentially carcinogenic and cellular DNA is constantly being damaged by electrophiles generated during the normal metabolism of foodstuffs, particularly fats. Next, there are those who think that the caloric restriction of laboratory rodents is unnatural and/or unkind. It is of course, nothing of the sort. Caged, bored animals, like frustrated humans, overeat for the want of anything more interesting to do. Those who run zoos or dog kennels know this very well. And those who have observed the effects of slight dietary restriction in rats and mice know that the animals are not stunted in terms of bone length or brain

size but are slimmer, brighter, more generally active and have healthier skins and hair than animals fed *ad lib.* Thirdly, there are those who have over many years collected data from studies in which animals have been fed *ad lib.* and realize that these collected data would be of little value if they switched to a calorie-restricted feeding regimen. The need to create and maintain databanks does not justify the perpetuation of bad science, and there can be no doubt that carcinogenicity tests conducted under conditions in which the results are hopelessly confused by the consequences of overfeeding constitute very bad science. Elderly overfed rats in very considerable endocrine disarray are most certainly not models suitable for detecting carcinogenic risk for humans. Untreated female rats of strains commonly used for routine carcinogenicity tests frequently exhibit close to 100% incidences of pituitary and mammary tumours. There is no sub-group of the human population that can match this. Slight diet restriction goes a long way to normalizing this situation. Thus, it is my view that, if long-term animal tests for carcinogenicity have a future, it must be one that involves animals that, if untreated, remain in normal physiological and hormonal status during the study period.

In the 1200-Rat Biosure Study (Roe, 1991) the full results of which are still being prepared for publication, we confirmed the findings reported previously in many other studies with regard to the effects of restricting calorie intake to 80% of *ad lib.* Some of the main findings are summarized in Table 7.

In thinking about the interpretation of these findings in relation to man, we tried to take into account the fact that there are no reliable data for the incidence of benign neoplasms in humans because of low autopsy rates. The only data available to epidemiologists—and these are seriously inaccurate (Heasman and Lipworth, 1966; Slavin *et al.*, 1991)—are cancer mortality data. Accordingly, we endeavoured to calculate cancer mortality rates for the rats. This involved assuming that malignant tumours found in animals that were killed for humane reasons caused the sickness for which they were killed. The effects of slight dietary restriction on cancer mortality were marked and highly statistically significant in both sexes (Roe and Lee, 1991) (Table 8). Moreover, the percentages of our calorie-restricted rats that developed potentially fatal malignant cancers (i.e. 38.7% of males and 35.5% of females) were not dissimilar from those in humans. At this point I should perhaps emphasize that these figures are for rats not deliberately exposed to any carcinogen—but simply fed on a standard laboratory diet.

The study as a whole involved several different diets and dietary regimens. When ignoring these differences, we found that the risks of premature death and of developing a malignant neoplasm were highly correlated with body weight 6 months after the start of the study (Table 9). Others have reported similar findings (e.g. Rao *et al.*, 1987).

We live in an age in which we are being bombarded by advice on what to eat and what not to eat. Claims and counter-claims as to what is riskier and what is safer are not infrequently based on laboratory experiments involving the feeding of qualitatively different diets. It needs to be said loudly and clearly that the results of research on which such claims are made are wholly unreliable unless conducted under isocaloric conditions. It is the total calorie intake and not dietary composition that is the main determinant of health risks from food in laboratory animals (Masoro, 1988; Weindruch and Walford, 1988).

Particularly pertinent in this regard are numerous investigations of the influence of diet on the development of mammary cancer in female rats previously exposed to a known potent carcinogen. Even if one can accept that rats given a large dose of a xenobiotic carcinogen (7,12-dimethylbenzanthracene) on about the 60th day of life are suitable models for studying breast cancer development in women, it makes a nonsense then to compare the effects of qualitatively different diets without controlling for calorie intake when one knows that calorie intake is a major determinant of whether and when mammary cancers will develop. A quotation from Ip (1991) makes the point quite clearly in relation to the DMBA-induced mammary tumour model: "Calorie restriction, even in the presence of a high fat intake, is more striking than a decrease in dietary fat in suppressing the development of induced mammary cancer".

The most obvious explanation of the relationships between calorie intake, premature ageing and cancer is that oxidants that damage both cell protein and DNA are generated during the metabolism of ordinary foodstuffs, particularly fats, and although efficient repair systems exist there is a slow build-up of unrepaired damage with the passage of time (Ames, 1989 and 1990; Ames and

Table 10. Effect of restricting calorie intake to 75% of *ad lib.* on cell turnover in the mouse

	% Inhibition of [³H]thymidine labelling index
Mammary gland	72++
Bladder epithelium	43+
Dermis	57++
Oesophageal epithelium	49++
Colon-crypt cells	54++

$+ = P < 0.05$; $++ = P < 0.01$.

(from Lok *et al.*, 1990)

Saul, 1987). There is a brief period in the cell cycle when the two strands of DNA are separated. During this brief period, DNA repair is hampered and there is an increased risk of damage being fixed and passed on to subsequent generations of cells. Cell-turnover rates are higher in overfed, that is *ad lib.* fed, rats and mice (Lok *et al.*, 1990) (Table 10) and consequently unrepaired DNA damage

and ageing-related damage to the structural components of cells accumulate more rapidly in them. During the last 2 years or so these concepts have been hotly debated, particularly in the columns of the journal *Science* (Abelson, 1990; Ames and Gold, 1990; Cohen and Ellwein, 1990; Weinstein, 1991), and the arguments are not over yet. Weinstein (1991) may be right in pointing out that the spectrum of DNA damage produced by oxidants released during the metabolism of food might well be different from that produced by xenobiotic genotoxic carcinogens. He may be right, too, in concluding that mitogenesis is only one factor in carcinogenesis. We still have to face the difficult problem of distinguishing the potentially more important damage to the DNA and proteins of stem cells and damage to non-stem cells. The temptation to conclude that carcinogenesis and ageing have the same cause needs to be tempered by the fact that most of the pathological changes associated with ageing diseases involve non-stem cells, which may or may not have abnormal DNA, whereas the whole edifice of modern day cancer research is built on the belief that genomic abnormality is invariably present in cancer cells.

Clearly, then, there is still a long way to go before we understand the relationship between calorie intake, premature ageing and cancer. Nevertheless, our present lack of understanding provides no excuse for ignoring data that point to calorie intake being a major determinant of the risk of developing all forms of cancer. Epidemiologists and toxicologists who fail to control for calorie intake in their studies are in danger of reaching false conclusions.

General conclusions

Although I have talked diversely on seven different topics, there is a theme that has run through them all. It is that damage to DNA by xenobiotic genotoxins is not the only determinant and perhaps not even the most important determinant, of whether and when a human or a rat develops cancer. There is at present no reason to suspect that mutations, usually multiple and successive, do not occur at some stage in cancer development, but they may well occur as a consequence of internally generated genotoxins during the metabolism of foodstuffs or in association with inflammatory processes. I have no doubt that necrosis followed by regenerative hyperplasia, particularly if this is a repeated sequence, increases the risk of cancer development. Against this background it would have been a great mistake to have banned the use of metronidazole because of its effects on bacteria *in vitro* or because in vast dosage it increased the incidence of certain commonly occurring tumours in overfed rodents.

Attention is drawn first to the gulf between cancer mortality data as used by epidemiologists and tumour incidence data as used by toxicologists, and secondly to the serious inaccuracy of human cancer mortality data. Partly for the latter reason, and more importantly because of poor exposure data and the difficulty of controlling for multiple confounding variables, epidemiologists can nei-

ther establish nor exclude low levels of cancer risk from exposure to factors such as environmental tobacco smoke. However, there is no excuse for experimentalists not to control for known confounders, such as calorie intake, in the course of tests for carcinogenicity in laboratory rodents.

References

Cameo I

Baker S.B. de C., Golberg L., Martin L.E. and Smith J.P. (1961) Tissue changes following injection of iron-dextran complex. *Journal of Pathology and Bacteriology*, **82**, 453–470.

Boyland E. (1958) The biological examination of carcinogenic substances. *British Medical Bulletin* **14**, 93–98.

British Medical Journal (1960) Carcinogenic risks of iron-dextran (Leading article). *British Medical Journal* **1**, 788–789.

Davies A.G., Beamish M.R. and Jacobs A. (1971) Utilization of iron dextran. *British Medical Journal* **1**, 146–147.

Golberg L., Martin L.E. and Smith J.P. (1960) Iron overloading phenomena in animals. *Toxicology and Applied Pharmacology* **2**, 683–707.

Golberg L. and Smith J.P. (1960a) Iron overloading and hepatic vulnerability. *American Journal of Pathology* **36**, 125–149.

Golberg L. and Smith J.P. (1960b) Vitamin A and E deficiencies in relation to iron overloading in the rat. *Journal of Pathology and Bacteriology* **80**, 173–180.

Golberg L., Smith J.J. and Baker S.B. de C. (1961) The significance of sarcomas induced in rats and mice by iron-dextran. *Biochemical Pharmacology* **8**, Abstract no. 233.

Greenberg G. (1976) Sarcoma after intramuscular iron injection. *British Medical Journal* **1**, 1508–1509.

Gross E. (1961) Uber die Erzeugung von Sarkomen durch die besonders gereinigten Triphenylmethanfarbstoffe Lichtgrun SF und Patentblau AE bei der widerholten subcutanen Injektion an der Ratte. *Zeitschrift für Krebsforschung* **64**, 287.

Haddow A. (1960) "Imferon" and cancer. *British Medical Journal* **2**, 663–664.

Haddow A. and Horning E.S. (1960) On the carcinogenicity of an iron-dextran complex. *Journal of the National Cancer Institute* **24**, 109–147.

Haddow A., Roe F.J.C. and Mitchley B.C.V. (1964) Induction of sarcomata in rabbits by intramuscular injection of iron-dextran ("imferon"). *British Medical Journal* **1**, 1593–1594.

MacKinnon A.E. and Bancewiez J. (1973) Sarcoma after injection of intramuscular iron. *British Medical Journal* **2**, 277–279.

Metcalfe J.B. (1976) Sarcoma after intramuscular iron injection. *British Medical Journal* **2**, 233–234.

Muir A.R. and Golberg L. (1961) The tissue response to iron–dextran; an electron-microscope study. *Journal of Pathology and Bacteriology* **82**, 471–482.

Richmond H.G. (1959) Induction of sarcoma in rats by iron–dextran complex. *British Medical Journal* **1**, 947–949.

Robertson A.G. and Dick W.C. (1977) Intramuscular iron and local oncogenesis. *British Medical Journal* **1**, 946.

Robinson C.E.G., Bell D.N. and Sturdy J.H. (1960) Possible association of malignant neoplasia with iron-dextran injection: a case report. *British Medical Journal* **2**, 648–650.

Roe F.J.C., Haddow A., Dukes C.E. and Mitchley B.C.V. (1964) Iron-dextran carcinogenesis in rats: effect of distributing injected material between one, two, four or six sites. *British Journal of Cancer* **18**, 801–808.

Cameo II

Gangolli S.D., Grasso P. and Golberg L. (1967) Physical factors determining the early local tissue reactions produced by food colourings and other compounds injected subcutaneously. *Food and Cosmetics Toxicology* **5**, 601–621.

Grasso P., Gangolli S.D., Golberg L. and Hooson J. (1971) Physicochemical and other factors determining local sarcoma production by food additives. *Food and Cosmetics Toxicology* **9**, 463–478.

Grasso P. and Golberg L. (1966a) Early changes at the site of repeated subcutaneous injection of food colourings. *Food and Cosmetics Toxicology* **4**, 269–282.

Grasso P. and Golberg L. (1966b) Subcutaneous sarcoma as an index of carcinogenic potency. *Food and Cosmetics Toxicology* **4**, 297–320.

Hooson J. and Grasso P. (1970) Inhibition of local connective tissue repair in the neoplastic response to water soluble carcinogen administered subcutaneously. *Experientia* **26**, 403–405.

Pott F., Ziem U., Reiffer F.-J., Huth F., Ernst H. and Mohr U. (1987) Carcinogenicity of studies on fibres, metal compounds and some other dusts. *Experimental Pathology* **32**, 129–152.

Cameo III

Beard C.M., Noller K.L., O'Fallon W.M., Kurland L.T. and Dockerty M.B. (1979) Lack of evidence for cancer due to use of metronidazole. *New England Journal of Medicine* **301**, 519–522.

Catterall R.D. (1977) Fifteen years experience with metronidazole. In *Metronidazole: Proceedings of the International Metronidazole Conference, Montreal, Quebec, Canada*. Edited by S.M. Finegold, J.A. McFadzean and F.J.C. Roe. p. 107. Excerpta Medica, Amsterdam.

Conybeare G. (1980) Effects of quality and quantity of diet on survival and tumour incidence in outbred Swiss mice. *Food and Cosmetics Toxicology* **18**, 65–75.

Cosar C. and Julou L. (1959) Activity of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (RP 8823) in experimental *Trichomonas vaginalis* infections. *Annales de l'Institut Pasteur* **96**, 238–241.

Despois R., Pinnert-Sindico S., Ninet L. and Preud'homme J. (1956) Three antibiotics of different groups produced by the same strain of streptomyces. *Journal of Microbiology* **21**, 76–90.

Drasar B.S., Hughes W.H., Williams R.E.O. and Shiner M. (1966) Bacterial flora of the normal intestine. *Proceedings of the Royal Society of Medicine* **59**, 1243.

Durel M.P., Roiron V., Siboulet A. and Borel L.J. (1959) Essai d'un limidazole: (8,923RP). Comptes rendus de la societé francaise de gynecologie **29**, 36–45.

Finegold S.M. (1977) *Anaerobic Bacteria in Human Disease*. Academic Press, London.

Friedman G.D. and Ury H.K. (1980) The initial screening for carcinogenicity of 53 commonly used drugs. *Journal of the National Cancer Institute* **65**, 723–731.

McFadzean J.A. (1986) *Flagyl: The Story of a Pharmaceutical Discovery*. pp. 1–117. Parthenon, Camforth, England.

Mitelman F., Hartley-Asp B. and Ursing B. (1976) Chromosome aberrations and metronidazole. *Lancet* **ii**, 802.

Nastro L.J. and Finegold S.M. (1972) Bacterial activity of five antimicrobial agents against Bacteroides fragilis. *Journal of Infectious Diseases* **126**, 104–107.

Roe F.J.C. (1977) Metronidazole: tumorigenicity studies in mice, rats and hamsters. Metronidazole: Proceedings of the International Metronidazole Conference, Montreal, Quebec, May 1976. pp. 132–137. Excerpta Medica, Amsterdam.

Roe F.J.C. and Tucker M. (1974) Recent developments in the design of carcinogenicity tests on laboratory animals. Excerpt a Medica International Congress Series **311**, 171–177.

Rosenkranz H.S. (1977) Bacterial mutagenicity studies. In Proceedings of the International Metronidazole Conference, Montreal, Quebec. Edited by S.M. Finegold, J.A. McFadzean and F.J.C. Roe. pp. 119–125. Excerpta Medica, Amsterdam.

Rust J.H. (1977) Tumorigenicity studies—mouse and rat. In Proceedings of the International Conference on Metronidazole. Montreal, Quebec. pp. 138–144. Excerpta Medica, Amsterdam.

Rustia M. and Shubik P. (1972) Induction of lung tumors and malignant lymphomas in mice by metronidazole. *Journal of the National Cancer Institute* **48**, 721–729.

Rustia M. and Shubik P. (1979) Experimental induction of hepatomas, mammary and other tumors with metronidazole in non-inbred sas:MRC(WI)BR rats. *Journal of the National Cancer Institute* **63**, 863–868.

Shinn D.L.S. (1962) Metronidazole in acute ulcerative gingivitis. *Lancet* **i**, 1191.

Study Group (1974) Metronidazole in the prevention and treatment of bacteriodes infections in gynaecological patients. *Lancet* **ii**, 1540–1543.

Study Group (1975) An evaluation of metronidazole in the prophylaxis and treatment of anaerobic infections in surgical patients. *Journal of Antimicrobial Chemotherapy* **1**, 393–401.

Voogd C.E., Van der Stel J.J. and Jacobs J.A. (1974) The mutagenic action of nitroimidazoles, metronidazole, nimorazole, dimetridazole and ronidazole. *Mutation Research* **26**, 483–490.

Willis A.T. (1977) *Anaerobic bacteriology. Clinical and Laboratory Practice*. 3rd Ed. Butterworths, London.

Cameo IV

Abramson J.H., Sacks M.I. and Cahana E. (1971) Death certificate as an indication of the presence of certain common diseases at death. *Journal of Chronic Diseases* **24**, 417–431.

Alderson M.R., Bayliss R.I.S., Clark C.A. and Whitfield A.G.W. (1983) Death certification. *British Medical Journal* **287**, 444–445.

Cameron H.M. and McGoogan E. (1981a) A prospective study of 1152 hospital necropsies. I. Inaccuracies in death certification. *Journal of Pathology* **133**, 273-283.

Cameron H.M. and McGoogan E. (1981b) A prospective study of 1152 hospital necropsies. II. Analysis of inaccuracies in clinical diagnosis and their significance. *Journal of Pathology* **133**, 285-300.

Cechner R.L., Chamberlain W., Carter J.R., Milojkovic-Mirceta L. and Nash N.P. (1980) Misdiagnosis of bronchogenic carcinoma—the role of cigarette smoking surveillance bias and other factors. *Cancer* **46**, 190-199.

Heasman M.A. and Lipworth L. (1966) Accuracy of certification of cause of death. General Register Office: Studies on medical and population subjects. No. 20. HMSO, London.

James G., Patton R.E. and Heslin A.S. (1955) Accuracy of cause-of-death statements on death certificates. *Bulletin of the New York Academy of Medicine* **51**, 432-438.

Lee P.N. (1993) Comparison of clinical and death certificate diagnosis with particular reference to lung cancer: a review of published data.

Mollo F., Bertoldo E., Grandi G. and Cavallo F. (1986) Reliability of death certification for different types of cancer. An autopsy survey. *Pathology Research and Practice* **181**, 442-447.

Peto R. (1974) Guidelines on the analysis of tumour rates and death rates in experimental animals. *British Journal of Cancer* **29**, 101-105.

Peto R., Pike M.C., Day N.E., Gray R.G., Lee P.N., Parish S., Peto J., Richards S. and Wahrendorf J. (1980) Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. *Monographs on the Evaluation of Carcinogenic Risks to Humans. Suppl. 2. Long-term and Short-term Screening Assays for Carcinogens: a Critical Appraisal.* pp. 311-426. International Agency for Research on Cancer, Lyon.

Report of the Joint Working Party of the Royal Colleges of Pathologists, Physicians and Surgeons (1991) The Autopsy and Audit. Published by Royal College of Pathologists, London, pp. 1-24.

Rosenblatt M.G., Teng P., Kerpe S. and Beck I. (1971) Cause of death in 1000 consecutive autopsies. *New York State Journal of Medicine* **71**, 2189-2193.

Steer A. and Land C.E. (1976) Accuracy of diagnosis of cancer among autopsy cases. JNIH-ABCC population for Hiroshima and Nagasaki. *Gann* **67**, 625-632.

Takeda K. and Kobayashi H. (1967). Reliability of cancer diagnosis in Japan. *Gann* **58**, 139-146.

Willis R.A. (1967) *Pathology of Tumours.* 4th Ed. pp. 67-91. Butterworths, London.

Wilson R.R. (1966) In defense of the autopsy. *Journal of the American Medical Association* **196**, 1011-1012.

World Health Organization (1992) World Health Statistics Annual 1991. WHO, Geneva.

Cameo V

Bailar J.C. (1989) Inhalation hazards: the interpretation of the epidemiologic evidence. In *Assessment of Inhalation Hazards.* Edited by U. Mohr, D.V. Bates, D.L. Dungworth, P.N. Lee, R.O. McClellan and F.J.C. Roe. pp. 39-48. Springer-Verlag, Berlin.

Chen B.H., Hong C.J., Pandey M.R. and Smith K.R. (1990) Indoor air pollution in developing countries. *World Health Statistics Quarterly* **43**, 127-138.

Clarke R.H. and Southwood T.R.E. (1989) Risks from ionizing radiation. *Nature* **338**, 197-198.

Davis B.R., Whitehead J.K., Gill M.E., Lee P.N., Butterworth A.D. and Roe F.J.C. (1975) Response of rat lung to inhaled tobacco smoke with or without prior exposure to 3,4-benzpyrene (BP) given by intratracheal installation. *British Journal of Cancer* **31**, 469-484.

Environmental Protection Agency (1992) Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders. US Environmental Protection Agency, Washington, DC.

Feron V.J., de Jong D. and Emmelot P. (1973) Dose-response for the induction of respiratory-tract tumours in Syrian golden hamsters by intratracheal instillations of benzo(a)pyrene. *European Journal of Cancer* **9**, 387-390.

Henry M.C., Port C.D., Bates R.R. and Kaufman D.G. (1973) Respiratory tract tumors in hamsters induced by benzo(a)pyrene. *Cancer Research* **33**, 1585-1592.

Hoffmann D. (1989) Nicotine, a tobacco specific precursor for carcinogens. In *Nicotine, Smoking and the Low Tar Programme.* Edited by N. Wald and P. Froggatt. pp. 24-40. Oxford Univesity Press, Oxford.

Holst P.A.J., Kromhout D. and Brand R. (1988) Pet birds as an independent risk for lung cancer. *British Medical Journal* **297**, 1319-1321.

Kohlmeier L., Arminger G., Bartolomeycik S., Bellach B., Rehm J. and Thamm M. (1992) Pet birds as an independent risk factor for lung cancer: case-control study. *British Medical Journal* **305**, 986-989.

Koo L.C., Ho J. H.-C. and Rylander R. (1988) Life history correlates of environmental tobacco smoke: a study on non-smoking Hong Kong Chinese wives with smoking versus non-smoking husbands. *Social Science and Medicine* **26**, 751-760.

Lee P.N. (1988) Misclassification of Smoking Habits and Passive Smoking: a Review of the Evidence. pp. 1-103. Springer-Verlag Berlin.

Lee P.N. (1992) *Environmental Tobacco Smoker and Mortality.* pp. 1-224. Karger, Basel.

Reynolds R.J. (1988) New cigarette prototypes that heat instead of burn tobacco: chemical and biological studies. pp. 743. R.J. Reynolds Tobacco Co., Winston Salem, NC.

Roe F.J.C. (1962) Role of 3,4-benzopyrene in carcinogenesis by tobacco smoke condensate. *Nature* **194**, 1089-1909.

Roe F.J.C. and Kearns F. (1967) Comparison of carcinogenicity of tobacco smoke condensate and particulate air pollutants and a demonstration that their effects may be additive. Alkylierend wirkende Verbindungen: Second Conference on Tobacco Research. pp. 110-111.

Roe F.J.C., Salaman M.H., Cohen J. and Burgan J.G. (1959) Incomplete carcinogens in cigarette smoke condensate: tumour promotion by a phenolic fraction. *British Journal of Cancer* **13**, 623-633.

Roe F.J.C. and Walters M.A. (1965) Some unsolved problems in lung cancer etiology. *Progress in Experimental Tumor Research* **6**, 126-227.

Shabad L.M. (1962) Experimental cancer of the lung. *Journal of the National Cancer Institute* **18**, 1305-1317.

Skrabanek P. (1990) Nonsense consensus. *Lancet* **335**, 1446-1447.

Whitehead J.K. (1977) An appraisal of various approaches to the fractionation of tobacco smoke condensate. Tobacco Research Council Research paper No. 13. pp. 1-56. Wynder

E.L., Graham E.A. and Croninger A.B. (1953) Experimental production of carcinoma with cigarette tar. *Cancer Research* 13, 855-864.

Cameo VI

Ames B.N. (1971) The detection of chemical mutagens with enteric bacteria. In *Chemical Mutagens: Principles and Methods for their Detection.* Edited by A.E. Hollaender. pp. 267-282. Plenum Press, New York. Ames B.N. (1983) Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science* 221, 1256-1264.

Ames B.N., Durston W.E., Yamasaki E. and Lee F.D. (1973) Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. *Proceedings of the National Academy of Sciences of the U.S.A.* 70, 2281-2285.

Ames B.N. and Saul R.L. (1987) Oxidative DNA damage. *Annals of Internal Medicine* 107, 526-545.

Biskind M.S. and Biskind G.R. (1944) Development of tumors of the rat ovary after transplantation into the spleen. *Proceedings of the Society of Experimental and Biological Medicine* 55, 176-179.

Cold Spring Harbor Laboratory (1987) *Nongenotoxic Mechnisms in Carcinogenesis.* Edited by B.E. Butterworth and T.J. Slaga. Banbury Report No. 25. pp. 397. Cold Spring Harbor, New York.

Gellatly J.B. (1975) The natural history of hepatic parendrynal nodule formation in a colony of C57BL mice with reference to the effect of diet. In *Mouse Hepatic Neoplasia.* Edited by W.H. Butler and P.M. Newberne. pp. 76-109. Elsevier, Amsterdam.

Hunter B., Graham C., Heywood R., Prentice D., Roe F.J.C. and Noakes D.N. (1978) Tumorigenicity and carcinogenicity study with xylitoe in long-term dietary administration to mice. Huntingdon Research Centre Report No. HLR/25/77774.

Roe F.J.C. and Baer A. (1985) Enzootic and epizootic adrenal medullary proliferative disease of rats: influence of dietary factors which affect calcium metabolism. *Human Toxicology* 4, 27-52.

Sinkeldam E.J., van Garderen-Hoetmer A. and Woutersen R.A. (1983) Life-span oral toxicity and carcinogenicity study with lactitol in rats pretreated in utero. CIVO-TNO Report No. V82.255.

Cameo VII

Abelson P.H. (1990) Testing for carcinogens with rodents. *Science* 249, 1357.

Ames B.N. (1989) Mutagenesis and carcinogenesis: endogenous and exogenous factors. *Environmental Mutagenesis* 13, 1-12.

Ames B.N. and Gold L.S. (1990) Too many rodent carcinogens: mitogenesis increases mutagenesis. *Science* 249, 970-971.

Ames B.N. and Saul R.L. (1987) Oxidative DNA damage. *Annals of Internal Medicine* 107, 526-545.

Berg B.N. and Simms H.S. (1960) Nutrition and longevity in the rat. *Journal of Nutrition* 71, 255-263.

Cohen S.M. and Ellwein L.B. (1990) Cell proliferation in carcinogenesis. *Science* 249, 1007-1011.

Conybeare G. (1980) Effects of quality and quantity of diet on survival and tumor incidence in outbred Swiss mice. *Food and Cosmetics Toxicology* 18, 65-75.

Conybeare G. (1988) Modulating factors: challenges to experimental design. In *Carcinogenicity: the Design, Analysis and Interpretation of Long-term Animal Studies.* Edited by H.C. Grice and J.L. Ciminera. Springer-Verlag, New York.

Deerberg F., Rapp K.G., Kaspareit-Rittinghausen J. and Lorcher K. (1990) The effect of food restriction by time-scheduled feeding on the development of body weight, lifespan and incidence of spontaneous tumours and diseases in male Hn:SPRD rats. *Zeitschrift für Versuchstierkunde* 33, 9.

Heasman M.A. and Lipworth L. (1966) Accuracy of certification of cause of death. General Register Office: Studies on Medical and Population Subjects No. 20. HMSO, London.

Ip C. (1991) The impact of calorie restriction on mammary cancer development in an experimental model. In *Biological Effects of Dietary Restriction.* Edited by L. Fishbein. pp. 65-72. Springer-Verlag, Berlin.

Lok E., Scott F.W., Mongeau R., Nera E.A., Malcolm S. and Clayson D.B. (1990) Calorie restriction and cell proliferation in various tissues of the female Swiss Webster mouse. *Cancer Letters* 51, 67-73.

McCay C.M., Crowell M.F. and Maynard L.A. (1935) Effect of retarded growth upon the length of life-span and upon ultimate body size. *Journal of Nutrition* 10, 65.

Masoro E.J. (1984) Nutrition as a modulator of the aging process. *Physiologist* 27, 98-101.

Masoro E.J. (1988) Minireview: Food restriction in rodents: an evaluation of its role in the study of aging. *Journal of Gerontology: Biological Sciences* 43, B59-B64.

Masoro E.J., Iwasaki K., Gleiser C.A., McMahan C.A., Seo E.U. and Yu B.P. (1989) Dietary modulation of the progression of nephropathy in aging rats: an evaluation of the importance of protein. *American Journal of Clinical Nutrition* 49, 1217.

Nolen G.A. (1972) Effect of various restricted dietary regimes on the growth, health and longevity of albino rats. *Journal of Nutrition* 102, 1477.

Rao G.N., Piegorsch W.W. and Haeman J.K. (1987) Influence of body weight on the incidence of spontaneous tumours in rats and mice of long-term studies. *American Journal of Clinical Nutrition* 45, 252.

Roe F.J.C. (1981) Are nutritionalists worried about the epidemic of tumours in laboratory animals? *Proceedings of the Nutrition Society* 40, 57.

Roe F.J.C. (1991) 1200-Rat Biosure Study: design and overview of the results. In *Biological Effects of Dietary Restriction.* Edited by L. Fishbein. pp. 287-304. Springer-Verlag, Berlin.

Roe F.J.C., Lee P.N., Conybeare G., Tobin T.G., Kelly D., Prentice D. and Matter B. (1991) Risks of premature death and cancer predicted by bodyweight in early adult life. *Human and Experimental Toxicology* 10, 285-288.

Roe F.J.C. and Lee P.N. (1991) Carcinogenicity tests. *Lancet* 337, 857.

Ross M.H. and Bras G. (1965) Tumour incidence patterns and nutrition in the rat. *Journal of Nutrition* 87, 245.

Ross M.H., Lustbader E.D. and Brs G. (1983) Dietary practices of early life and age at death of rats with tumours. *Journal of the National Cancer Institute* 71, 947.

Salmon G., Leslie G.N., Roe F.J.C. and Lee P.N. (1990) The influence of longevity, organ weights and the incidence of non-neoplastic and neoplastic diseases in rats. *Food and Chemical Toxicology* 28, 39-48.

Simms H.S. and Berg B.N. (1962) Longevity in relation to lesion onset. *Geriatrics* 17, 235-242.

Slavin G., Kirkham N., Underwood J.C.E., Holt J.M., Hopkins A., Jackson B.T. and Winsor C.W.O. (1991) The autopsy and audit: Report of a Joint Working Party of the Royal Colleges of Pathologists, Physicians and Surgeons. pp. 1-24.

Tannenbaum A. (1940) Relationship of body weight to cancer incidence. *Archives of Pathology* **30**, 509.

Tannenbaum A. (1942) The genesis of growth of tumours II. Effect of caloric restriction *per se*. *Cancer Research* **2**, 460.

Tucker M.J. (1979) Effects of long term diet restriction on tumours in rodents. *International Journal of Cancer* **23**,803.

Turnbull G.J., Lee P.N. and Roe F.J.C. (1985) Relationship of body-weight gain to longevity and to risk of development of nephropathy and neoplasia in Sprague-Dawley rats. *Food and Chemical Toxicology* **23**, 355-361.

Weindruch R.H. and Walford R.H. (1988) *Retardation of aging and disease by dietary restriction.* pp. 436. Charles C. Thomas, Springfield, IL.

Weinstein I.B. (1991) Mitogenesis is only one factor in carcinogenesis. *Science* **251**, 387-388.

Yu B.P., Masoro E.J., Murata I., Bertand H.A. and Lynd F.T. (1982) Life span study of SPF Fischer 344 male rats fed *ad libitum* or restricted diets: longevity, growth, lean body mass and disease. *Journal of Gerontology* **37**, 130-141.

MYCOTOXINS IN THE DIET

The Steering Group on Chemical Aspects of Food Surveillance has published its third report on mycotoxins, which it last reviewed in 1987 (*BIBRA Bull.* 1987, **26**, 117).

Patulin in apple juice, which has been the subject of much press comment, was found at higher levels than previously. This was probably due to the growing popularity of fresh, cloudy juices, often produced from fruit held in long-term storage. Some 17 samples of cloudy juices contained concentrations in the range to 434 mg/kg, with a median value of 28 mg/kg. In 15 clear juice samples concentrations ranged only up to 118 mg/kg, with a median value below 10 mg/kg (the detection limit), and in all but one sample were below 50 mg/kg. Apple juice manufacturers have now agreed to adhere to GMP by avoiding the use of mouldy fruit, and a follow-up survey is planned.

The proportion of whole dried figs exceeding the new statutory limit for aflatoxins of 4 mg/kg (*ibid* 1993, **31**, 55) fell from 26% to 16% between December 1988 and April 1992, although a high maximum level of 427 mg/kg was found during the last sampling year. The proportion of fig pastes exceeding the limit likewise declined from 50% to 14% over the same period, and in this case the maximum level also fell, from 165 to 15 mg/kg. Some 28% of pistachio nuts sampled during 1991–92 contained more than 4 mg/kg, and 25% exceeded the 10 mg/kg limit for products that are to be further processed, the maximum detected being 813 mg/kg. All consignments found to contain more than 10 mg/kg were refused entry by port authorities, and none of 109 retail samples from the Norwich area proved to contain more than 4 mg/kg. Discussions with fig and nut importers and the Turkish and Iranian producers have now taken place to improve control measures, including screening and analysis before shipment.

'Crunchy' peanut butter continued to contain more aflatoxin than 'smooth' varieties, and the proportion of both types containing more than 4 mg/kg rose between 1986 and 1991, from 11% to 31% for crunchy and from 10% to 19% for smooth varieties, although maximum concentrations remained similar (53 mg/kg in 1986 and 51 mg/kg in 1991). It was concluded that industry will need to change its sorting procedures to ensure consistent compliance with the new regulations. Aflatoxin B_1 levels in animal feedingstuffs consistently complied with the applicable regulations (*ibid* 1988, **27**, 344), and aflatoxin M_1 levels in milk and cheese presented no cause for concern.

Ochratoxin A concentrations ranged up to 45 mg/kg in cereals, 9.3 mg/kg in pig kidney and 1.8 mg/kg in black pudding, although only 10–29% of different products sampled contained more than the detectable limit of 1 mg/kg. Assuming that each foodstuff contained the maximum level found for that sample type, it was estimated that extreme consumers might ingest 9.5 ng/kg body weight/day. This is below the JECFA provisional tolerable weekly intake of 112 ng/kg body weight (*ibid* 1990, **29**, 293; *ibid* 1991, **30**, 150 & 334), which, however, was based on the no-effect level for kidney damage and did not take account of subsequent evidence of genotoxicity. Ochratoxin A has recently been reported in Swedish human blood plasma samples, and a survey of UK blood is now planned.

Moniliformin was found in all but three of 36 maize products (whole maize, meal and flour) sampled in 1990, at levels in the range 50 to 250 mg/kg. As certain ethnic groups consume unprocessed maize as a dietary staple, periodic surveillance will continue, although its priority will be low.

In comments on the report the CoT welcomes the new aflatoxin regulations, and recommends that surveillance of affected products should continue. As ochratoxin A has mutagenic, carcinogenic, teratogenic and immunotoxic effects and is persistent within the body, contamination should be reduced to the lowest level technologically achievable. Moniliformin produces myocardial degeneration in rats, for which a no-effect level has not been established, and is suspected to contribute to Keshan disease (cardiomyopathy) in China. As few of the UK population are likely to consume unprocessed maize products regularly, the levels found are not considered to be of particular concern, but the effects of processing should be investigated, since processed maize products are common items in the UK diet. Patulin is mutagenic and has adverse reproductive, immunological, neurological and gastro-intestinal effects in rodents, and levels should therefore be reduced as much as possible. Surveillance should be extended to other food products containing

apples, and continued to ensure that effective control measures are introduced by industry. In future surveys it should be ascertained whether there are other important fungal metabolites in the UK diet, the toxicity and incidence of which require further investigation.

This advice is endorsed by the FAC, who also recommend that the apple juice industry should introduce quality control measures to keep patulin below an action level of 50 mg/kg. A code of practice for apple juice production should be drawn up, advisory material on the dangers of patulin should be prepared, targeting in particular the smaller producers of apple juice, and the public should be warned not to use mouldy fruit in home juice production.

[Mycotoxins: third report. The thirty-sixth report of the Steering Group on Chemical Aspects of Food Surveillance. MAFF Food Surveillance Paper No. 36. HMSO, London, 1993, pp. xii + 66. £9.00.]

ABSTRACTS FROM THE LITERATURE

Cola drinks
A time-related increased incidence of DNA damage (adducts) was found in the liver of mice given cola drinks in place of drinking water for 8 wk. Myristicin, a naturally occurring compound found in several foods including nutmeg and mace, was identified as the major component responsible. DNA adducts were also detected in foetal liver when pregnant mice were intubated with myristicin (Randerath *et al., Biochemical and Biophysical Research Communications* 1993, **192**, 61).

Caffeine
The effect of exposing pregnant rats to caffeine on the reproductive parameters of their offspring and outcome through eight subsequent litters has been studied by Australian investigators. Female rats (F_0) received either 30 or 60 mg caffeine/kg body weight daily by stomach tube from day 2 to 21 of pregnancy. Female offspring (F_1) of these animals were then bred through eight consecutive matings. The F_1 rats derived from mothers given the top dose of caffeine bled heavily when giving birth to the F_2 generation, and there was a dose-related increase in the number of stillborn litters. Birth weight was often increased in the F_2 offspring, most notably in the first litter (Pollard and Claassens, *Reproductive Toxicology* 1992, **6**, 541).

Formaldehyde resins
US investigators examined all records for patients at two dermatitis clinics between January 1988 and April 1990 (a total of 1022 patients) to determine the prevalence of positive patch-test reactions to formaldehyde-based textile resins (used in 'permanent press clothing') and the clinical and demographic patterns associated with textile resin allergy. 17 patients with allergy to formaldehyde textile resins were identified. Interestingly, in five of these cases, allergy to formaldehyde itself could not be demonstrated (Fowler, Jr *et al., Journal of the American Academy of Dermatology* 1992, **27**, 962).

Styrene
Performance in a number of psychological tests was poorer in 30 French dockyard workers with daily exposure (for a mean period of 5 yr) to low levels of styrene than in 30 unexposed carefully matched control workers. Subjects were tested on Monday morning and again in the evening following a daily exposure to an average 23 ppm (range 4 to 55 ppm). Exposed workers performed less well than controls both in the morning and evening tests. However, there was no indication of a deterioration in performance within the group following the day's exposure, nor did those with 8-14 yr of exposure perform significantly worse than those with only 1 yr of exposure. The current UK maximum exposure limit for styrene is 100 ppm (8-hr TWA) (Jégaden *et al., International Archives of Occupational and Environmental Health* 1993, **64**, 527).

Dinitrotoluene
An increased risk of cancer of the liver and biliary tract for munitions workers exposed to dinitrotoluene has been reported by US investigators. The six observed cases among the 4989 exposed workers represented rate ratios for hepatobiliary cancer of 2.67 or 3.88 in comparison with either the US population or a group of 7436 unexposed workers at the same plant, respectively. Subjects had worked at the study plant for at least 5 months between 1949 and 1980. No relationship between duration of exposure and increased risk of developing hepatobiliary cancer was observed (Stayner *et al., Journal of Occupational Medicine* 1993, **35**, 291).

Antimony
US investigators have suggested that the current occupational exposure limit for antimony is not adequate to prevent skin disease, after reporting the cases of three workers who developed dermatitis following exposure to antimony dust and antimony trioxide fumes. Evaluation of the work process, which included breaking up and melting antimony ingots revealed an 8-hr TWA for antimony of 0.39 mg/m³, although for one 250-min period the average concentration was 0.67 mg/m³. The current UK occupational exposure standard for antimony is 0.5 mg/m³ (8-hr TWA) (White, Jr *et al., Journal of Occupational Medicine* 1993, **35** 392).

Cancer and parental drug use
An increased incidence in children of the rare cancer rhabdomyosarcoma was associated with parental use of marijuana and cocaine. In a US case-control study comparing 322 cases (aged 0–20 years) with 322 controls, mother's marijuana use during the year before their child's birth was associated with a three-fold increased risk of disease, while cocaine use was associated with a 5.1-fold increase. Father's marijuana or cocaine use were each associated with around two-fold increases (Grufferman *et al., Cancer Causes and Control* 1993, **4**, 217).

[Philip Copestake —BIBRA]