Methods for Assessing the Effects of Mixtures of Chemicals
Edited by V. B. Vouk, G. C. Butler, A. C. Upton, D. V. Parke and S. C. Asher

Mechanisms of Interaction and Modulation of Response

Paul M. Newberne

1 INTRODUCTION

There is a large body of literature describing the many environmental factors that modulate the response of humans and other mammalian species to environmental toxins and carcinogens, most of which are encountered in mixtures. Many factors or conditions are encountered routinely in daily life by the human population; some are under individual control, others not. Table 1 illustrates the numerous aspects of modern-day living that impinge on our responses to mixtures of chemicals over which we do have some control.

One of the most striking examples of environmental effects is diet. The following pages will fully indicate that environmental factors, many of which are under voluntary control, contribute to risk of chronic disease. These factors are confounding elements but provide a basis for planning strategies and approaches to the predictive evaluation of multiple exposure to chemicals.

Table 1 Intrinsic and extrinsic factors contributing to risk of chronic disease in humans

A. Intrinsic factors
1. Dietary deficiencies
2. Hormonal factors
3. Metabolic deficiencies
4. Internal chemicals
B. Extrinsic factors
1. Life-style
(a) Alcohol
(b) Tobacco
(c) Diet
2. Air pollution
3. Food and water contaminants
4. Industrial exposure

2 NUTRITION AND CARCINOGENESIS

Diet and nutritional status may influence susceptibility to cancer induction in a number of ways. Dietary contaminants in the food chain (Newberne and McConnell, 1980a,b) result in exposure to carcinogens and procarcinogens of a number of types (Table 2). Interactions between nutrients and toxic substances occur in the diet and after ingestion; these interactions can either enhance or inhibit a toxic or carcinogenic effect. Nutritional status influences the activation or deactivation of chemicals through a modulating effect on microsomal or cytosolic enzyme systems. In addition, nutrients have a profound effect on the immune system, influencing the body's surveillance mechanisms. There can be little doubt that environmental influences, particularly diet, play a role in the response of both humans and animals to toxic materials (NAS, 1982).

It is generally accepted that some natural substances (e.g. aflatoxin) are human carcinogens; in concert with hepatitis B virus, aflatoxin is probably the major cause of primary liver cancer in some areas of the world (Okuda *et al.*, 1982; Peers and Linsell, 1973). Food additives may contribute to human cancer in some small way but the overall contributions from this source are probably not significant.

It is well established from studies in animals that nutrients can affect the impact of some additives. Furthermore, it is clear that some of the macronutrients (e.g. fats) act as promoters in animal carcinogenesis. It is also accepted that nutrients such as vitamins E and C influence the formation of nitrosamines in foods (Mirvish, 1983) or *in vivo* in biological systems and in this way may modulate the incidence of some types of human cancer. One of the most common food additives, table salt (sodium chloride), is associated with gastric cancer in Japan, perhaps by producing injury to the gastric mucosa and thus permitting increased exposure to nitrosamines or other carcinogens (Stemmermann *et al.*, 1977).

Table 2 Contaminants found in natural product diets

Toxin	Dietary content, various lots					
Aflatoxin B_1 (ppb)	10	200	120	4	21	80
Nitrosamines (ppb)						
N-Dimethylnitrosamine	8	32	18	5	83	12
N-Nitrosopyrrolidine	7	16	5	2	11	22
Nitrates (ppm)	23	41	180	5	90	3
PCBs (ppm)	9	3	28	15	23	5
Arsenic (ppm)	0.3	0	3	1	4	2
Lead (ppm)	0.8	4.2	3.2	1.0	0.2	2
Cadmium (ppm)	0.5	1.2	0.9	2.0	1.3	0.4
Mercury (ppm)	0.7	1.2	0.5	0.3	0.8	1.0

Each figure represents a different lot from the same manufacturer. Not all diets contained all listed chemicals. From Newberne and McConnell (1980a, b). *Abridged, by permission.*

2.1 Calories

2.1.1 Epidemiological Evidence

Epidemiological data regarding the effect of calories on cancer incidence do not permit quantitation of total dietary intake, and thus total caloric intake cannot be accurately determined. Many of the dietary studies in human populations have been based on preselected food lists. The international distribution of hormone-dependent cancers has generated suspicion that these types of tumours may be related to affluence and in this way related to an overall total calorie effect (Berg, 1975). Hill *et al.* (1979) have suggested that affluence doubles the mortality from colorectal cancer in some human populations. Mortality rates for gastric cancers fall as the per capita food intake increases but for intestinal cancer the rates rise, perhaps reflecting effects of a longer lifespan in the populations where increased per capita food intake is associated with decreased gastric cancer, allowing more time for large bowel cancer to develop.

Doll and Peto (1981) and Gaskill *et al.* (1979) have reported a positive effect of calories on cancer incidence but others have failed to find such an effect (Jain *et al.*, 1980; Miller *et al.*, 1978).

In long-term prospective studies (Lew and Garfinkel, 1979) the American Cancer Society has shown that cancer mortality was significantly elevated in both sexes only among those that were 40 % or more overweight and that males under similar weight excess had increased mortality from cancer of the colon and rectum. Women, in addition to greater risk for breast cancer with increased overweight, also had an increased risk for endometrial cancer.

2.1.2 Animal Studies

Tannenbaum (1942a,b, 1944, 1945a,b) showed that tumours induced by benzo[*a*]pyrene in mice were inhibited by caloric restriction. Similar effects were observed by this investigator with dimethylbenzanthracene (DMBA). Mice with a daily dietary intake of 11.7 calories had 25 % more spontaneous mammary tumours than mice with a daily caloric intake of 9.6 calories.

2.2 Protein and Amino Acids

2.2.1 Epidemiological Evidence

Kolonel *et al.* (1981), Armstrong and Doll (1975), and others have reported correlations between total protein intake and a number of forms of cancer (Gaskill *et al.*, 1979; Gray *et al.*, 1979; Gregor *et al.*, 1969; Hems, 1978, 1980). Others have not confirmed such an association (Bingham *et al.*, 1979; Jain *et al.*, 1980).

2.2.2 Animal Studies

An inhibitory effect of selected amino acid deficiencies on tumour responses in laboratory animals was reported as early as 1936 (Voegtlin and Maver, 1936; Voegtlin and Thompson, 1936). Generally, animals that have been fed lower dietary concentrations of protein have developed fewer tumours. Since several factors in addition to protein were being varied at the same time it is difficult to interpret the results of some of these studies. It should be noted also that in some studies the levels of carcinogen varied between the high and the low dietary protein groups of animals (Babson, 1954). Furthermore, the total food intake was less for animals fed very high levels of protein.

There are a number of significant reports relative to the relationship of dietary protein to chemically induced tumours in animal models. If aflatoxin is fed in diets with varying levels of protein, the incidence of liver tumours is generally lower in the animals fed the diets with lower concentrations of protein. Table 3, taken from Madhavan and Gopalan (1968), illustrates a typical effect. Young rats intubated with a carcinogenic dose of aflatoxin and then fed either 5% or 20% casein diets for one year clearly showed the effects of protein. The rats fed 5% protein had no tumours whereas 50% of those fed the 20% casein diet had tumours. Wells *et al.* (1976) and Temcharoen *et al.* (1978) essentially confirmed the work of Madhavan and Gopalan.

In the case of aflatoxin B_1 a protein effect is related to the rate of activation of the parent compound by the mixed function oxidase enzyme system to the 2,3-epoxide which binds to DNA, and, while less well understood, in some degree to repair of DNA.

A number of studies have been conducted in attempts to define the mechanism of how protein may influence carcinogenesis. This is unclear at present but probably is an indirect effect acting via the mixed function oxidase enzyme systems which are responsible for metabolism of carcinogens such as aflatoxin B_1 (Campbell, 1979; Campbell *et al.*, 1978; Mgbodile and Campbell, 1972; Preston *et al.*, 1976). Taken together, the evidence from both epidemiological and laboratory studies only suggests that protein may have an effect on risk of cancer

Table 3 Effect of dietary protein on aflatoxin

Carcinogenesis treatment: aflatoxin, plus	No. rats with tumours
High-protein diet	11/30
Low-protein diet	0/12

From Madhavan and Gopalan (1968). *Abridged, by permission.*

of certain sites but the lack of adequate data on this important dietary ingredient precludes firm conclusions.

2.3 Carbohydrates

2.3.1 Epidemiological Evidence

When fibre is excluded and considered separately from other carbohydrates there is very little in the literature relative to this class of dietary ingredients and their effect on cancer. The epidemiological and experimental evidence is not convincing.

2.3.2 Animal Studies

A single experimental study relative to carbohydrate and experimental cancer in animals offers some interesting data. Gershoff and McGandy (1981) examined the interaction of dietary lactose fed at a level of 49 %, or sucrose varying from 43 % to 55 % of the diet and vitamin A deficiency. Urinary bladder tumours in Charles River rats were associated with bladder stones induced by the high-lactose diet.

2.4 Fibre

The real or potential effects of fibre as a type of carbohydrate are sufficiently different from those of simple carbohydrates to require separate consideration. The physiological significance of dietary fibre is still in question as to an effect on response to toxins and carcinogens. It is now recognized that fibres differ markedly in digestibility and in the chemical constituency such as cellulose, lignin, haemacellulose, pectins, and gums. However, a major characteristic common to all of these is the capacity to form bulk in the gastrointestinal tract.

2.4.1 Epidemiological Evidence

Most of the data derived from epidemiological studies have been directed toward the possible role of dietary fibre in protection against large bowel cancer. The proposed mechanisms have ranged broadly from the capacity of fibre to dilute carcinogens that are present in the large bowel, to speeding intestinal transit time of ingesta, to influencing composition and metabolic activity of the microflora of the gut, the latter involved in the production of putative carcinogens by modification of faecal bile acids. At best, the epidemiological studies have yielded conflicting results (Drasar and Irving, 1973; Liu *et al.*, 1979).

In a study involving adult males from Denmark who are at a high risk for colon cancer and comparing them to a group from Finland, a low-risk

population, it was found that the Danes consumed less fibre and stool weights were much less than those of the Finns (MacLennan *et al.*, 1978). Bingham *et al.* (1979), in studies in the United Kingdom, found no significant correlation between total fibre intake and mortality rates from colorectal cancer (Bjelke, 1978; Dales *et al.*, 1978; Martinez *et al.*, 1979).

2.4.2 Animal Studies

There have been a relatively large number of studies done in animal models in attempts to correlate fibre and colon cancer using chemical carcinogens.

Some reports (Table 4) suggest that bran has some protective effect in rats against dimethylhydrazine-induced colon cancer (Barbolt and Abraham, 1978; Chen *et al.*, 1978; Wilson *et al.*, 1977). Freeman and coworkers have also observed a protective effect by cellulose when fed to rats also exposed to dimethylhydrazine (DMH) (Freeman *et al.*, 1980). Other studies have been conducted, the results of which are equivocal in many cases and conflicting in others (Fleiszer *et al.*, 1980; Watanabe *et al.*, 1978, 1979).

2.5 Dietary Lipids

The influence of quality and quantity of dietary fats has been studied from both epidemiological and experimental standpoints, probably more than any other single dietary ingredient. Moreover there is probably more convincing evidence regarding fat and its relation to breast cancer than there is for any of the other nutrients. Despite the fact that there have been large numbers of studies, it is

Table 4 Fibre, dimethylhydrazine (DMH) and colon tumours in rats

Treatment	Percentage colon tumours	Tumours per tumour-bearing rat
4 Doses DMH		
Beef fat, no bran	68	1.9
Beef fat, plus bran	38	1.1
Corn oil, no bran	65	1.5
Corn oil, plus bran	43	1.6
8 Doses DMH		
Beef fat, no bran	70	3.0
Beef fat, plus bran	63	3.1
Corn oil, no bran	63	3.1
Corn oil, plus bran	66	3.4

From Wilson *et al.* (1977). *Abridged, by permission.*

sometimes difficult to interpret the findings because protein is almost always an accompanying nutrient and it is difficult to say that the observed effects are in fact a result of fat alone.

2.5.1 Epidemiological Evidence

A number of studies have shown direct associations between the per capita intake of fat and breast cancer incidence or mortality (Armstrong and Doll, 1975; Berg, 1975; Doll and Peto, 1981; Gaskill *et al.*, 1979; Phillips, 1975). In most of these studies the correlations have been higher for total fat than for other dietary factors considered at the time, including animal protein, meat, or specific fat components in oils. These data are strong for populations but weak for individuals.

Kolonel *et al.* (1981) correlated the consumption of fat with breast cancer incidence in Hawaii and found that there were significant associations between breast cancer and total fat consumption, with animal fat, and with both saturated and unsaturated fats. Phillips (1975) and Phillips *et al.* (1980) observed a direct association between the frequency of consumption of high-fat foods in breast cancer in a case–control investigation among Seventh-day Adventists in California. Lubin *et al.* (1981), Miller *et al.* (1978), and Nomura *et al.* (1978), using case–control studies, have also associated the consumption of fat with breast cancer in a positive way.

2.5.2 Animal Studies

Experimental evidence for an effect by dietary fat on mammary carcinogenesis, either spontaneous or chemically induced, was reported more than 40 years ago by Tannenbaum (1942b). The incidence of spontaneous tumours in mice was greater when the high-fat diets were started at 24 weeks of age compared with waiting until the animals were 38 weeks of age, nearing half of their lifespan. Furthermore, Tannenbaum (1942b, 1944, 1945b) has shown that fat, rather than calories *per se*, was responsible for enhancing tumorigenesis by feeding mice isocaloric high- and low-fat diets.

The quality as well as the quantity of fat were shown (Carroll, 1980) to be important factors in the induction of breast cancer as was the carcinogen and its dose. This investigator concluded that dietary fat exerts its effect during the promotional phase of carcinogenesis and that unsaturated fat promoted tumours more than saturated forms of lipids. Studies in our own laboratory (Rogers and Wetsel, 1981; Wetsel *et al.*, 1981) and by others suggest that it is the type of fat that determines whether or not it influences carcinogenesis during exposure to the chemical or afterwards (initiation or promotion stages of tumorigenesis). In most of the studies reported to date it appears that the amount of unsaturated fatty acids, particularly linoleic acid, in the fat under

consideration has a significant effect on the response of animals to chemical carcinogenesis.

Additional studies in animal models have shown that the effects of a high-fat diet on breast carcinogenesis can be modified, particularly by diets that are marginal in the lipotropic factors, choline and methionine (Newberne and McConnell, 1980a,b; Rogers and Newberne, 1980).The tumour incidence induced by AFB_1 was lower (Table 5) and the death rate from tumours occurred later in DMBA-treated rats if the diet was marginal in lipotropic factors, compared with those fully supplemented.

Dietary fat can modify hepatocellular carcinomas induced in rats by aflatoxin B_1. When beef fat was fed to rats, the number of tumours was the same irrespective of when the beef fat was fed, either during the period of exposure to the carcinogen or before and after exposure to the carcinogen (Newberne *et al.*, 1979). On the other hand, feeding polyunsaturated fat before and after carcinogen exposure resulted in 100% tumour yield compared with only 66% tumours when the oil was fed only after tumour induction. It was concluded in this study that unsaturated fats increased tumour yield more effectively than saturated fats and that this effect may occur during the initiation or early promotional phase of hepatocarcinogenesis.

Experiments in our laboratory, using both DMH (which requires metabolic activation) and NMU (a direct-acting carcinogen) to induce colon tumours in

Table 5 Effects of dietary fat, during or after exposure to a carcinogenic dose of aflatoxin B_1 (AFB_1), and tumour incidence

Group number	Dietary treatment and AFB_1 exposure[a]				After 14 months		
	Beef fat		Corn oil		Incidence of liver tumours		Incidence of lung metastases
	During treatment with AFB_1	After treatment with AFB_1	During treatment with AFB_1	After treatment with AFB_1	No.	%	
1	+	+	0	0	32	53	25.0
2	0	+	0	0	28	51	21.0
3	0	0	+	+	60	100	60.3
4	0	0	0	+	38	66	36.8

[a] Prior to feeding these diets, rats were fed a diet as follows (%): casein, 20; sucrose, 15; dextrose, 14; Rogers–Harper salts mix, 5; vitamin mix, 2; lard, 4; corn oil, 2; beef fat, 4. The vitamin mix was complete for rats. All rats received crystalline aflatoxin B_1. From Newberne *et al.* (1979). *Reproduced by permission of* Cancer Research.

rats, have failed to support the hypothesis that quality and quantity of fat in our experimental systems do indeed influence carcinogenesis (Locniskar *et al.*, 1983).

With regard to dietary fat and cancer it would appear at this point that the evidence for an effect of dietary fat in both experimental and epidemiological studies is greatest for breast cancer. There is also evidence for dietary fat having an influence on prostate cancer in men and, perhaps, uterine cancer in women. The data on large bowel cancer, however, are equivocal and do not provide convincing evidence at this point that fats have an influence on colon cancer.

2.6 Vitamins

A major current interest in regard to diet, nutrition and cancer is centred on some of the vitamins (Newberne *et al.*, 1977, 1983). In particular, lipotropes, vitamin A and the retinoids, vitamin C, and vitamin E have come under intense study.

2.6.1 Vitamin A

2.6.1.1 Epidemiological Evidence One of the first investigators to report the correlation between vitamin A and lung cancer was Bjelke (1975). This investigator studied Norwegian men and observed lower values for vitamin A in lung cancer cases than in controls, after controlling for cigarette smoking. Additional studies by Gregor *et al.* (1980) and MacLennan *et al.* (1977, 1978), while varying somewhat in assessment, indicated in both cases that those with lung cancer had consumed less vitamin A than controls. Others have also pointed out that lung cancer incidence has varied inversely with carotene intake and with foods that contain higher concentrations of vitamin A (Mettlin *et al.*, 1979; Shekelle *et al.*, 1981; Smith and Jick, 1978). A similar finding with urinary bladder cancer has been reported (Mettlin *et al.*, 1981). This has also been indicated in cancer of the larynx (Graham *et al.*, 1972); the oesophagus (Cook-Mozaffari *et al.*, 1979; Mettlin *et al.*, 1981) and the stomach (Bjelke, 1978). More recent cohort studies in the United Kingdom and in the United States have suggested an inverse relationship between serum levels of vitamin A and cancer risk in general (Shamberger, 1971; Wald *et al.*, 1980).

2.6.1.2 Animal Studies It has been shown that if rats are maintained on a diet deficient in vitamin A, neoplastic lesions induced by MCA (methylcholanthrene) are enhanced resulting in the appearance of urinary bladder tumours in a shorter period of time. The same applies for colon tumours (Suphakarn *et al.*, 1983).

A number of studies regarding vitamin A and in particular the synthetic retinoids have been reported by Sporn *et al.* (1976) and Sporn and Newton (1979, 1981) to have a protective effect against a number of animal tumours. We have shown in our laboratory (Newberne and McConnell, 1980a,b) that vitamin A

Table 6 Vitamin A, 13-*cis*-retinoic acid and lung cancer in the Syrian golden hamster

Treatment (diet)	Malignant tumours of respiratory tract	
	Number	%
Control, 2 μg/g retinyl acetate, BP	46/89	51.7
Low vitamin A, 0.3 μg/g retinyl acetate, BP	102/127	80.3
High vitamin A, 30 μg/g retinyl acetate, BP	40/88	45.4
Control, 2 μg/g retinyl acetate + 13-*cis*-retinoic acid during dosing BP	38/83	45.8
Control, 2 μg/g retinyl acetate + 13-*cis*-retinoic acid during and after dosing BP	4/91	4.4
Control, 2 μg/g retinyl acetate + 13-*cis*-retinoic acid after dosing BP	11/84	13.1

From Newberne and McConnell (1980b). *Reproduced by permission of Barry A. Scherr.*

deficiency enhances but 13-*cis*-retinoic acid inhibits lung cancer in hamsters (Table 6).

2.6.2 Vitamin C (Ascorbic Acid)

It was early in the 1960s when reports appeared in the literature noting that consuming foods rich in ascorbic acid was inversely related to the appearance of certain types of cancer. Shortly thereafter there were other reports that suggested that vitamin C protected against gastric cancer, perhaps by blocking the reaction of secondary amines with nitrite to form *N*-nitroso compounds, some of which are gastric carcinogens (Mirvish, 1981, 1983; Mirvish *et al.*, 1972).

2.6.2.1 Epidemiological Evidence An inverse association between oesophageal cancer and the consumption of fresh fruits which contain ascorbic acid (Cook-Mozaffari *et al.*, 1979) was reported for humans along the Caspian Sea. More

recently, another report has appeared (Wassertheil-Smoller *et al.*, 1981) which indicates an inverse relationship between vitamin C consumption and uterine cervical dysplasia in women in New York. On the other hand, Jain *et al.* (1980) failed to find an association between ascorbic acid consumption and colon cancer in a case–control study.

2.6.2.2 Animal Studies It has been shown conclusively that ascorbic acid can prevent nitrosation of amines which in turn prevents the formation of nitrosamines, some of which are gastric carcinogens (Mirvish, 1981; Mirvish *et al.*, 1972). Soloway *et al.* (1975), among others, have found no effects of vitamin C. It must be remembered, however, that this class of compounds is only one of several which are associated with gastric cancer. Banic (1981) observed that vitamin C acted as a cocarcinogen to MCA.

Based on evidence available to date one can only suggest that vitamin C or ascorbic acid may have an influence on human cancer, but in animal systems it has been established only that it inhibits carcinogenesis through its effect on nitrosamine formation. Other considerations require further elucidation.

2.6.3 Vitamin E

2.6.3.1 Epidemiological Evidence There is no epidemiological evidence that vitamin E has an effect on tumour induction in humans.

2.6.3.2 Animal Studies Vitamin E or α-tocopherol can have an influence on tumours induced in experimental animals (Shklar, 1982) and there is some indication that vitamin E acts the same as ascorbic acid doses in blocking the formation of nitrosamines (Mergens *et al.*, 1979; Wattenberg, 1972).

A number of investigators have studied the effects of α-tocopherol on DMBA-induced mammary tumours in animals (Wattenberg, 1972). The ingestion of high levels of α-tocopherol during the initiation stage of mammary tumours had no effect. Shamberger *et al.* (1973, 1976) reported an inhibiting effect of vitamin E on skin tumours in mice promoted by croton oil. Haber and Wissler (1962) noted that there was a marked decrease in the carcinogenicity of MCA in mice if the animals were given diets containing supplemental vitamin E.

2.7 Minerals

Of the essential trace element nutrients, only two (selenium and zinc) will be discussed here. Cadmium, arsenic and lead, as poisonous heavy metals, will also be briefly considered (Furst, 1979).

2.7.1 Selenium

2.7.1.1 Epidemiological Evidence Selenium deficiency and toxicity have been economic problems in livestock for decades. It was in the 1930s when a deficiency

was clearly identified as the cause of important livestock diseases referred to as 'white muscle disease' (NAS, 1971). The work that led to a much better understanding of selenium and its significance to animal and possibly human disease was not done until the 1950s (Shamberger and Willis, 1971; Shamberger *et al.*, 1973, 1976).

Shamberger and colleagues have reported on selenium levels in the blood of 100 cancer patients and compared these with the selenium content in the blood of 48 normal subjects (Shamberger *et al.*, 1973). The analyses revealed that patients with gastrointestinal cancer and Hodgkin's disease had significantly lower levels of selenium than normal subjects but there were no differences between normal subjects and patients with cancer at other sites.

Another group of workers (Schrauzer, 1976, 1979; Schrauzer *et al.*, 1977a,b) have correlated per capita intake of selenium with mortality rates in more than 20 countries. These reports were based on disappearance rates of major staple foods rather than actual estimates of food intake. The blood levels of selenium and corresponding cancer mortality rates in 22 different countries were compared; these revealed an inverse relationship between selenium levels and most cancers at various sites.

2.7.1.2 Animal Studies Results of experimental animal studies have been controversial and conflicting. Studies that were conducted by the Food and Drug Administration (FDA) during the 1940s indicated that high levels of selenium induced or enhanced tumour formation (Nelson *et al.*, 1943). In addition, a series of studies from Russia (Volgarev and Tscherkes, 1967) reported, two decades later, that animals given high levels of selenium supplements in the diet developed liver cirrhosis and hepatocellular carcinoma. There are a number of major defects in both of these studies; the FDA investigators used diets that were low in protein and, to compound the stress, they used a protein of low quality. The difficulty in the interpretation of the Russian studies was related to the fact that a selenium-free control group was not used.

Results of both of these studies appear to have been influenced by extraneous dietary factors or conditions unrelated to selenium.

Studies in our laboratories (Newberne and Conner, 1974; Newberne and McConnell, 1980a,b) have shown that either a deficiency or an excess of selenium can adversely influence the acute or chronic response of rats to aflatoxin B_1 (Table 7) (Newberne, 1974). The chronic studies were terminated after 12 months, insufficient to induce tumours at the low dose of carcinogen (total 50 μg of aflatoxin B_1). Nevertheless, even at this low dose and short duration, tumours developed in livers damaged by the 5 ppm level of selenium, a hepatotoxic dietary concentration. A more recent study (unpublished) indicated that a deficit or an excess enhances aflatoxin B_1 cancer but that 2 ppm selenium is protective. Other investigators have reported a protective role for selenium in rodents exposed to a variety of carcinogens (Greeder and Milner, 1980; Ip and Sinha, 1981; Jacobs

Table 7 Influence of selenium on aflatoxin B_1 (AFB_1) carcinogenesis

Group	Treatment (ppm)		Liver injury	Nodular hyperplasia	Hepatocellular carcinoma
	AFB_1	Selenium			
1	–	0.05	0	0	0/20
2	+	0.10	0	0	0/20
3	+	0.50	0	0	0/20
4	+	1.00	0	0	0/20
5	+	2.00	0	1/20	0/20
6	+	3.50	+	3/20	0/20
7	+	5.00	+	20/20	14/20

Aflatoxin B_1 was administered after 8 weeks on diet, 5 daily doses of 10 μg each. Rats were sacrificed at the end of 12 months. From Newberne and McConnell (1980b). *Reproduced by permission of Barry A. Scherr.*

et al., 1981; Medina and Shepherd, 1980; Medina *et al.*, 1983; Schrauzer *et al.*, 1978; Thompson and Becci, 1980).

The animal data regarding selenium and cancer strongly suggest an effect of this trace element on the induction of cancer in animal models, but the limited evidence does not permit firm conclusions.

2.7.2 Zinc

Zinc is essential to more than 100 mammalian enzyme systems, and it has been demonstrated to be effective in reversing certain types of diseases in human populations (Prasad, 1978). Its role in animal nutrition has been established for many decades. It is also accepted that severe zinc deficiency does occur in humans and more moderate forms of deficiency are not uncommon (Prasad, 1978).

2.7.2.1 Epidemiological Evidence The levels of zinc in blood and other body tissues have been examined in cancer patients and compared with controls by a number of investigators. Schrauzer *et al.* (1977a,b) observed that the mean zinc concentration in food and in blood correlated directly with corresponding mortality rates from cancer of the large bowel, breast, ovary, lung, bladder, and oral cavity. Zinc levels in the blood were inversely correlated with each of these. Strain *et al.* (1972), in examining patients with bronchogenic carcinoma, observed that zinc levels differed between the cancer patients and age-matched controls. Copper levels were also lower in controls resulting in high ratios of zinc to copper in cancer patients. Davies *et al.* (1968) had reported earlier that the levels of zinc in the plasma of bronchogenic carcinoma patients were lower than those of other patients with other types of cancer and were also lower than normal laboratory values.

Studies from our own laboratory (Lin *et al.*, 1977) have shown that the levels of zinc in serum, hair, and from the diseased but non-cancerous oesophageal tissue from oesophageal cancer patients were significantly lower than levels in patients with other types of cancer, in patients with other types of disease, or in normal subjects. The concentration of zinc in the hair was lower in all cancer patients than in normal subjects. In agreement with the observation of Strain *et al.* (1972), our work (Lin *et al.*, 1977) found significantly elevated copper levels in oesophageal cancer patients, affecting the zinc: copper ratios. Furthermore, the serum of such patients had much lower iron levels than normal subjects.

2.7.2.2 Animal Studies The studies of Petering *et al.* (1967), who worked with transplanted Walker 256 carcinoma in rats, showed that zinc deficiency inhibited the growth of transplanted tumours in animals and also prolonged the survival time. These data were confirmed by DeWys *et al.* (1970) and DeWys and Poires (1972), where the effects of zinc were extended to leukaemia, lung carcinoma, and plasmacytoma TEPC-183 (Fenton *et al.*, 1980). Studies conducted in our own laboratory using the oesophagus as the tumour target organ (Fong *et al.*, 1978; Gabrial *et al.*, 1982) have consistently shown an enhancing effect of zinc deficiency on nitrosamine-induced oesophageal cancer and an inhibitory effect if supplements were administered at levels of nutritional requirements.

Poswillo and Cohen (1971) have reported that a high level of dietary zinc, greatly exceeding the nutritional requirements, suppressed the carcinogenic effect of DMBA in hamsters. Additional evidence for an effect of excess zinc was reported also by Duncan and Dreosti (1975).

From the foregoing conclusions it seems clear that the epidemiological evidence is not yet available to make specific comments on the effects of zinc in human cancer, but in experimental animal models it does have a profound effect, the results of the studies depending on the tissue examined and the animal model used in the investigation.

3 TOXIC HEAVY METALS

3.1 Cadmium

3.1.1 Epidemiological Evidence

Berg and Burbank (1972) observed direct associations between cadmium levels and mortality from myeloma, lymphoma, and cancer of the oral cavity and upper neck area, including the oesophagus. In addition, cadmium exposure was associated with cancer of the breast, lung, and large intestine as well as with the urinary bladder. These data were based on mortality from cancer by state, in the United States, and the trace element content of the water supplies.

In a case–control study where the combined exposure to cadmium from three different sources was evaluated, it was reported that cadmium contained in the

diet, in cigarette smoke, and in the workplace was associated with an increased risk of renal cancer (Kolonel, 1976). A number of observations have been reported, based on occupational exposure, which have indicated a relationship between cadmium and prostatic cancer in men (Adams *et al.*, 1969; Kipling and Waterhouse, 1967; Lemen *et al.*, 1976). This was not confirmed by a later case–control study (Kolonel and Winkelstein, 1977).

3.1.2 Animal Studies

In experimental carcinogenesis studies the effect of dietary cadmium on carcinogenicity has been reported from only one long-term study (Schroeder *et al.*, 1964, 1965) and this has not been confirmed. There are, however, a number of reports on the mutagenicity of cadmium and on various sources of cadmium, most of which support a mutagenic role for this element (Casto *et al.*, 1976; Shiraishi *et al.*, 1972; Sirover and Loeb, 1976; Yagi and Nishioka, 1977).

3.2 Arsenic

3.2.1 Epidemiological Evidence

The consumption of water contaminated with arsenic has been associated with an increased risk of skin cancer in some areas of the world (Tseng, 1977; Tseng *et al.*, 1968). Vineyard workers in Germany and in France are reported to suffer from a higher incidence of cancers of the skin, lung, and liver, associated with clinical evidence of chronic arsenical poison (Galy *et al.*, 1963; Latarjet *et al.*, 1964; Roth, 1957). Others (Tsuchiya, 1977) have failed to find an effect.

3.2.2 Animal Studies

Baroni *et al.* (1963) reported no effect of arsenic, as 0.01 % of drinking water, given to Swiss mice. Furthermore, skin painting studies and exposure through drinking water in other laboratories have been negative (Boutwell, 1963; Hueper and Payne, 1962). All other attempts to induce cancer in animals with arsenic have been negative (Byron *et al.*, 1967; Casto *et al.*, 1976; Nordenson *et al.*, 1978; Petres *et al.*, 1970, 1977).

Arsenic is one of the few chemicals associated with cancer in humans for which we do not have an animal model that reproduces experimental cancer. This suggests in part that there are other cofactors involved in the human observations which have not been revealed in the laboratory as yet.

3.3 Lead

3.3.1 Epidemiological Studies

Lead has been directly correlated with cancer of the stomach, small intestine, large intestine, ovary, and kidney, as well as with myeloma and all forms of

leukaemia (Berg and Burbank, 1972). Nelson *et al.* (1973), on the other hand, found that there was no increased risk of cancer mortality in a population that consumed apples from orchards treated with lead arsenate. Most of the studies on occupational exposures to lead have shown no association between lead and any form of cancer (Dingwall-Fordyce and Lane, 1963; Robinson, 1976). Cooper and Gaffy (1975) have reported on a study which is difficult to interpret but the results might be construed as being either positive or negative; thus, it does not add to epidemiological evidence for a significant role for lead in cancer and human populations (IARC, 1980; Kang *et al.*, 1980).

3.3.2 Animal Studies

In contrast to human populations it has been shown by VanEsch and Kroes (1969) that mice fed 0.1 % lead subacetate had a significant increase in renal tumours compared with their untreated controls. In addition, Wistar rats fed lead acetate have exhibited more renal tumours than their untreated controls (Boyland *et al.*, 1962; Mao and Molnar, 1967; Shakerin and Paloucek, 1965; VanEsch *et al.*, 1962). In most of the feeding studies rats exposed to lead in the diet have developed renal tumours (Boyland *et al.*, 1962; Hass *et al.*, 1967; Ito, 1973; Ito *et al.*, 1971).

Lead acetate was negative in the Ames test (Rosenkranz and Poirier, 1979) and in the host-mediated assay in Swiss Webster mice (Simmon *et al.*, 1979). In most other reported studies lead has, in fact, been negative for mutagenicity even though some studies have shown chromosome abnormalities including chromatid breaks (Teodorescu and Calugaru, 1972). Sister chromatid exchange in human leukocytes was not affected by lead (Beek and Obe, 1974, 1975).

The animal experimentation suggests a role for lead in some types of cancer but the epidemiological evidence in human populations is lacking.

4 OTHER CONTAMINANTS

In this review contaminants will be considered in the broadest sense to include those factors that we take intentionally or which are added unintentionally to our diet, as well as those that find their way into the food chain unintended by human populations.

4.1 Intentional Contaminants

Probably no other class of chemicals added to foods has created more controversy in recent years than the non-nutritive sweeteners, saccharin, cyclamate, and aspartame (Morrison and Buring, 1980). These three will briefly be considered in this review as they may relate to human risks of cancer.

4.1.1 Saccharin

There have been a number of epidemiological studies relative to saccharin and bladder cancer (Hoover and Strasser, 1980; IARC, 1980) primarily directed at the possible effects in diabetics who earlier on were the primary users of saccharin. The results did not indicate a direct association between saccharin and bladder cancer.

Furthermore, studies in England and Wales and in other countries have failed to show an increased bladder cancer mortality (Morgan and Jain, 1974; Simmon *et al.*, 1979). The work of Arnold *et al.* (1977, 1980) has established that saccharin is an animal carcinogen.

4.1.2 Cyclamates

Cyclamic acid, sodium cyclamate, and calcium cyclamate were also used as non-nutritive sweeteners in the United States until 1970 when they were banned (USDA, 1978). Much of the use was as a mixture of 10 parts cyclamate to 1 part saccharin used in dry beverage bases, diet foods, sweetener formulations, and carbonated beverages (Wiegand, 1978).

The fact that cyclamates were largely used in combination with saccharin made it a difficult matter to determine just what effect cyclamates *per se* might have on biological systems. Apparently for this reason there is no epidemiological evidence on cyclamates alone that indicates they are carcinogenic.

In animal systems, particularly in mice, cyclamates have been largely negative in regard to carcinogenic effects (Branton *et al.*, 1973; Homburger, 1978; Kroes *et al.*, 1977; Roe *et al.*, 1970). Some rather bizarre experiments have been conducted in which very potent carcinogens have been applied along with or prior to the feeding of sodium cyclamate (Chowaniec and Hicks, 1979; Hicks *et al.*, 1978). In lifetime studies under such unusual experimental design there was a significantly higher incidence of bladder cancer and a significant decrease in the latent period compared with animals treated with NMU only. These kinds of data are unconvincing as to the carcinogenicity of cyclamates and, in fact, are misleading.

4.1.3 Aspartame

Aspartame, the newest addition to the FDA-approved non-nutritive sweeteners, is a methyl ester of the amino acids phenylalanine and aspartic acid. It is approximately 180 times sweeter than sugar (NAS, 1978). After almost a decade of arguments between and among the manufacturer, the FDA, and consumer advocates as to the validity of safety evaluation data, the FDA finally approved the use of aspartame as a sweetener or flavouring agent in certain foods in 1981.

There are no convincing data that aspartame is carcinogenic in animals (Ishii *et al.*, 1981; Searle & Co., 1974a,b).

Aspartame is also negative for mutagenicity (Searle & Co., 1974a, b) using the Ames test with and without S9 fraction in rats and using the host-mediated assay in rats and in mice (Searle & Co., 1974b). Based on all of the evidence available to date, aspartame is not carcinogenic or mutagenic in any of the animal species used in studies conducted to this time.

4.1.4 Antioxidants

It is only from some unusual experimental designs that either BHA (butylated hydroxyanisole) or BHT (butylated hydroxytoluene) has shown effects suggestive of carcinogenicity (NCI, 1979). The effect has been mainly in the promotion of tumours induced by other known carcinogens (Witschi, 1981). In addition some studies have indicated that BHT may have some mutagenic activity (Trosko *et al.*, 1982). Both BHA and BHT, used according to standard protocols, have anticarcinogenic activity, probably through their capacity to scavenge lipid peroxy radicals or 'active' oxygen formed during prostaglandin synthesis (USFDA, 1977).

4.1.5 Diethylstilboestrol

Diethylstilboestrol (DES) is one of about 20 growth hormones that have been used in animal feed to promote increased animal protein production. DES is the one compound that has been examined in greatest detail for carcinogenicity primarily because it was reported to be carcinogenic in animals as early as 1964 (Fitzhugh, 1964). An additional cause for concern (Cutler *et al.*, 1972) has been related to the administration of DES to human patients for the prevention of menstrual disorders and miscarriages, and as chemotherapy for prostate cancer in men and breast cancer in women. These practices were allowed until 1978 when they were banned mainly because of the occurrence of vaginal tumours in young women born to mothers who used DES during pregnancy (Greenwald *et al.*, 1973; Herbst and Cole, 1978).

DES fed to mice increases the incidence of mammary cancers (Gass *et al.*, 1974). Rats fed DES for a major portion of their lifespan developed pituitary tumours in excess over controls and an increase in liver and mammary tumours. The progeny of pregnant Syrian golden hamsters which had been administered DES by intubation exhibited a high incidence of neoplastic lesions of the genital tract (Rustia, 1979).

DES was not mutagenic in the Ames test (Glatt *et al.*, 1979) although chromosome aberrations and other positive results have been reported in some systems (Chrisman, 1974; Ivett and Tice, 1981; Martin *et al.*, 1978).

Both the data in humans and the data in animals clearly indicate that DES is a carcinogen.

4.1.6 Other Food Additives

There are many other intentional food additives including an estimated 12 000 chemicals which are used in various aspects of food processing, packaging and distribution which must be included here, although only about 3000 substances are intentionally added to processed foods (Flavor and Fragrance Materials, 1981). Since these food additives including those that impart colour, flavour, or taste to foods are added in such minute quantities, their testing in animals or studies in people is virtually impossible in terms of realistic concentrations under conditions of use. Furthermore, there has been no evidence provided to date that the rather marked increase in use of food additives over the past three or four decades has significantly contributed to the overall risk of cancer in humans.

4.2 Non-intentional Contaminants

These substances include trace elements and organometallic compounds, natural and synthetic radioactive substances, and natural or synthetic organic compounds.

4.2.1 Pesticides

Pesticide residues often remain on agricultural commodities after they have been prepared for consumer purchase. Furthermore, they are also found in processed foods derived from commodities used for human consumption. Despite the long and widespread exposure of large human populations to pesticides there is very little reliable information about real or potential effects on human health. The three major types of pesticides are the organochlorines, the organophosphates, and the carbamates, all of which are commonly detected in the diet of humans but generally at levels one or two orders of magnitude below the acceptable daily intake (IARC, 1974). Of the three classes, the organochlorine compounds are metabolized slowly and accumulate in body tissues (USFDA, 1977). In addition, organochlorines and organophosphates have the potential for modulating the activity of microsomal enzymes and through this mechanism they affect in some way either the activation or the deactivation of other chemicals in the environment. Some of these chemicals produce either benign or malignant tumours in experimental animals and must be viewed with some concern. However, it is also reasonable to predict that the amounts that are present in the average US diet do not make a major contribution to human cancer risk (Barthel, 1976; IARC, 1974; USEPA, 1975, 1979, 1980; Wang and Grufferman, 1981; Wang and MacMahon, 1979).

With the exception of parathion (NCI, 1979), the organophosphates do not cause cancer in laboratory animals. However, a number of the organochlorine

pesticides do cause rodent tumours (Cabral *et al.*, 1979; Innes *et al.*, 1969; NCI, 1977, 1978a, b; Thorpe and Walker, 1973).

4.2.2 Benzo[a]anthracene

Benzo[*a*]anthracene has been found in the average American diet but there are no reports indicating an effect of this compound on human cancer. It has been shown, however, that the administration of benzo[*a*]anthracene to mice produces papillomas of the forestomach, liver and lung adenomas (Bock and King, 1959; Klein, 1963) and further that it is mutagenic in the Ames test and positive in the DNA repair tests (Hollstein *et al.*, 1979; Moryeau *et al.*, 1976). Benzo[*a*]anthracene thus is carcinogenic when administered orally to rodents.

4.2.3 Benzo[a]pyrene

This compound has been established as an animal carcinogen by numerous investigators. When administered intragastrically it produces forestomach tumours in mice (Peirce, 1961). In the diet it also produces forestomach tumours, lung tumours, and leukaemia (Rigdon and Neal, 1969). Sprague-Dawley rats have increased incidences of mammary tumours, and hamsters fed benzo[*a*]pyrene developed oesophageal, intestinal, and stomach tumours (Chu and Malmgren, 1965; Huggins and Yang, 1962). Benzo[*a*]pyrene has also been shown to be mutagenic in a number of systems (Hollstein *et al.*, 1979; Nagao and Sugimura, 1978; Tong *et al.*, 1981).

4.2.4 Polychlorinated Biphenyls (PCBs)

This class of chemicals consists of complex mixtures of chlorinated hydrocarbons and has been in use for well over 50 years (IARC, 1978).

The major route of exposure to low levels of PCBs for humans is dietary exposure. They have been identified in cheese, eggs, fish, milk, and animal feed derived from animal products such as fish meal (Jelinek, 1981). PCBs have also been detected in human tissues (Kutz and Strassman, 1976) and limited epidemiological data (Brown and Jones, 1981) suggest that exposure to high levels may be associated with malignant melanomas (Bahn *et al.*, 1976; Kuratsune, 1976; Kuratsune *et al.*, 1976). However, no conclusion can be drawn about the varied low levels that occur in contaminated foods. It is now considered more likely that PCBs may act primarily as tumour-promoting agents (Kimura *et al.*, 1976) based in part on their capability for inducing microsomal enzyme systems associated with the activation of carcinogens. On the other hand, it has been shown that one of the PCBs (Aroclor 1254) induces dose-related hepatocellular carcinoma in female rats and enhances other types of induced liver cancer (Kimbrough and Linder, 1974; Kimbrough *et al.*, 1978, 1981; Preston *et al.*, 1981).

4.2.5 Polybrominated Biphenyls (PBBs)

This class of chemicals is related to PCBs and has been used as flame retardants in a number of industrial processes. As with PCBs, PBBs persist in the environment and accumulate in body fat.

The most extensive exposure to PBBs is that which occurred in Michigan in 1973; the exposure was associated with a number of adverse effects on health (Kay, 1977). The time interval between exposure and the measurement of effects, however, has been short and these studies did not provide definitive information about the relationship between PBBs and cancer (Kimbrough *et al.*, 1978, 1981).

4.2.6 Mycotoxins

These natural contaminants will be covered in other sections of the proceedings and will be only briefly alluded to here. The family, particularly members of the aflatoxin group, contaminate foods of both man and other animals (Newberne, 1974) and, as such, constitute a health hazard and a source of modification of response to other chemicals. The mycotoxins to which man and animals are exposed include aflatoxins, mainly B_1, sterigmatocystin, ochratoxin A, zearalenone, T-2 toxin, patulin, penicillic acid, griseofulvin, luteoskyrin, cyclochlorotine, and ergot. Aflatoxin B_1 appears to be the most important; a consistent body of evidence, based on correlational data, associates the contamination of foods by aflatoxin B_1 with a high incidence of liver cancer in parts of Africa and Asia. Epidemiological studies have also indicated a high correlation between primary hepatocellular carcinoma and exposure to hepatitis B viral infection, but aflatoxin is likely also involved. Aflatoxin is carcinogenic in several species of animals, including rats, mice, trout, ducks, monkeys, and marmosets; generally, in a dose–response fashion, it induces mainly tumours of the liver and, to a lesser extent, tumours in the kidney, lung, stomach, and colon, more readily in males and in the young. The carcinogenicity of aflatoxin is paralleled by its mutagenicity in various systems (NAS, 1982; Newberne, 1974). There is no reliable information about the role of other mycotoxins in carcinogenesis in humans.

4.2.7 Nitrosamines

Epidemiological evidence (Shank, 1981) suggesting that *N*-nitroso compounds play a role in the development of cancer in humans is largely circumstantial. However, the findings from several epidemiological studies of certain geographical/nationality groups are consistent with the hypothesis that the exposure of humans to high levels of nitrate and/or nitrite may be associated with an increased incidence of cancers of the stomach and oesophagus. In these studies, the level, duration, and time of exposure were not studied in relation to cancer incidence, and exposure to other known or suspected carcinogens was not excluded.

In animals, nitrate has not been shown to be carcinogenic or mutagenic *per se*. The data on nitrite, while suggestive (Newberne *et al.*, 1979), indicate that nitrite is probably not carcinogenic other than as a nitrosating agent, but that it is mutagenic.

5 CONCLUSIONS

The list of factors and conditions which can modify a response to environmental toxins and carcinogens is long and continues to grow daily as our knowledge expands in this important area of toxicology. High on the list is life-style, with particular interest in diet and nutrition. Deficiency, and sometimes an excess, of essential dietary nutrients can predispose human and lower animal populations to environmental toxicants. Vitamins A and C, total calories, protein and amino acids, lipotropes, selenium, and zinc have been associated with enhancement or inhibition of neoplasia in human and other species. Toxic heavy metals (lead, cadmium, arsenic) have all been associated with animal toxicity and some forms of human neoplasia and, aside from arsenic, carcinogenicity has been demonstrated in experimental animals. Aflatoxin has now been declared a human carcinogen by IARC.

Some of the intentional and non-intentional exposures are more recent and their effects are less well understood because of the short time frame over which the exposure has occurred. Intentional food additives would appear to be relatively safe, but aflatoxins, nitrosamines, pesticides, PAH, and PCB and PBB compounds are of significance as toxic or carcinogenic agents and they modify responses to other environmental contaminants.

In terms of improvements in methodologies for evaluation of environmental toxicants, research animals should be free of disease, housed properly, and given a diet free of toxicants or, at a minimum, containing known quantities, and containing proper amounts and ratios of essential nutrients. Test animals should not be permitted to become obese, and certain dietary modifications might be considered to increase the sensitivity of animals to chemical toxins.

6 REFERENCES

Adams, R. G., Harrison, J. F., and Scott, P. (1969). The development of cadmium-induced proteinuria, impaired renal function, and osteomalacia in alkaline battery workers. *Q. J. Med.*, **38,** 425.

Armstrong, B., and Doll, R. (1975). Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *Int. J. Cancer*, **15,** 617.

Arnold, D. L., Moodie, C. A., Stavric, B., Stoltz, D. R., Grice, H. C., and Munro, I. C. (1977). Letter to the editor. Canadian saccharin study. *Science*, **197,** 320.

Arnold, D. L., Moodie, C. A., Grice, H. C., Charbonneau, B., Stavric, B., Collins, B. T., McGuire, P. F., Zamidzka, Z. Z., and Munro, I. C. (1980). Long-term toxicity of ortho-toluenesulfonamide and sodium saccharin in the rat. *Toxicol. Appl. Pharmacol.*, **52,** 113.

Babson, A. L. (1954). Some host-tumour relationships with respect to nitrogen. *Cancer Res.*, **14,** 89.

Bahn, A. K., Rosenwaike, N., Hermann, P., Grover, P., Stellman, J., and O'Leary, K. (1976). Letter to the editor. Melanoma after exposure to PCBs. *N. Engl. J. Med.*, **295,** 450.

Banic, S. (1981). Vitamin C acts as a cocarcinogen to MCA in guinea pigs. *Vitam. Horm.* (NY), **22,** 591.

Barbolt, T. A., and Abraham, R. (1978). The effect of bran on dimethylhydrazine-induced colon carcinogenesis in the rat. *Proc. Soc. Exp. Biol. Med.*, **157,** 656.

Baroni, C., VanEsch, G. C., and Saffiotti, V. (1963). Carcinogenesis tests in two inorganic arsenicals. *Arch. Environ. Health*, **7,** 668.

Barthel, E. (1976). High incidence of lung cancer in persons with chronic professional exposure to pesticides in agriculture. *Z. Erkr. Atmungsorgane*, **146,** 266.

Beek, B., and Obe, G. (1974). Effect of lead acetate on human leukocyte chromosomes *in vitro*. *Experientia*, **30,** 1006.

Beek, B., and Obe, G. (1975). The human leukocyte test system. VI. The use of sister chromatid exchanges as possible indicators for mutagenic activities. *Humangenetik*, **29,** 127.

Berg, J. W. (1975). Can nutrition explain the pattern of international epidemiology of hormone-dependent cancers? *Cancer Res.*, **35,** 3345.

Berg, J. W., and Burbank, F. (1972). Correlations between carcinogenic trace metals in water supplies and cancer mortality. *Ann. N.Y. Acad. Sci.*, **199,** 249.

Bingham, S., Williams, D. R. R., Cole, T. J., and James, W. P. T. (1979). Dietary fibre and regional large-bowel cancer mortality in Britain. *Br. J. Cancer*, **40,** 456.

Bjelke, E. (1974). Letter to the editor. Colon cancer and blood-cholesterol. *Lancet*, **i,** 1116.

Bjelke, E. (1975). Dietary vitamin A and human lung cancer. *Int. J. Cancer*, **15,** 561.

Bjelke, E. (1978). Dietary factors and the epidemiology of cancer of the stomach and large bowel. *Aktuel. Ernaehrungsmed. Klin. Prax. Suppl.*, **2,** 10.

Bock, F. G., and King, D. W. (1959). A study of the sensitivity of the mouse forestomach toward certain polycyclic hydrocarbons. *J. Natl. Cancer Inst.*, **23,** 833.

Boutwell, R. K. (1963). A carcinogenicity evaluation of potassium arsenite and arsenilic acid. *J. Agric. Food Chem.*, **11,** 381.

Boyland, E., Dukes, C. E., Grover, P. L., and Mitchley, C. V. (1962). The induction of renal tumours by feeding lead acetate to rats. *Br. J. Cancer*, **16,** 283.

Branton, P. B., Gaunt, I. F., and Grasso, P. (1973). Long-term toxicity of sodium cyclamate in mice. *Food Cosmet. Toxicol.*, **11,** 735.

Brown, D. P., and Jones, M. (1981). Mortality and industrial hygiene study of workers exposed to PCBs. *Arch. Environ. Health*, **36,** 120.

Byron, W. R., Bierbower, G. W., Brouwer, J. B., and Hansen, W. H. (1967). Pathological changes in rats and dogs from two-year feeding of sodium arsenite or sodium arsenate. *Toxicol. Appl. Pharmacol.*, **10,** 132.

Cabral, J. R. P., Mollner, T., Raitano, F., and Shubik, P. (1979). Carcinogenesis of hexachlorobenzene in mice. *Int. J. Cancer*, **23,** 47.

Campbell, T. C. (1979). Influence of nutrition on metabolism of carcinogens. *Adv. Nutr. Res.*, **2,** 29.

Campbell, T. C., Hayes, J., and Newberne, P. M. (1978). Dietary lipotropes, hepatic microsomal mixed function oxidase activities, and *in vivo* covalent binding of aflatoxin B_1 in the rat. *Cancer Res.*, **38,** 4569.

Carroll, K. K. (1980). Lipids and carcinogenesis. *J. Environ. Pathol. Toxicol.*, **3(4),** 253.

Casto, B. D., Pieczynski, W. J., Nelson, R. L., and DiPaolo, J. A. (1976). *In vitro* transformation and enhancement of viral transformation with metals. *Proc. Am. Assoc. Cancer Res. Am. Soc. Clin. Oncol.*, **17,** 12.

Chen, W. F., Patchefsky, A. S., and Goldsmith, H. S. (1978). Colonic protection from dimethylhydrazine by a high fiber diet. *Surg. Gynecol. Obstet.*, **147,** 503.

Chowaniec, J., and Hicks, R. M. (1979). Response of rat to saccharin with particular reference to the urinary bladder. *Br. J. Cancer*, **39,** 355.

Chrisman, C. L. (1974). Aneuploidy in mouse in embryos induced by diethylstilboestrol diphosphate. *Teratology*, **9,** 229.

Chu, E. W., and Malmgren, R. A. (1965). An inhibitory effect of vitamin A on the induction of tumours of forestomach and cervix in the Syrian hamster by carcinogenic polycyclic hydrocarbons. *Cancer Res.*, **25,** 884.

Cook-Mozaffari, P. J., Azordegan, F., Day, N. E., Ressicand, A., Sabai, C., and Aramesh, B. (1979). Oesophageal cancer studies in the Caspian littoral of Iran: results of a case–control study. *Br. J. Cancer*, **39,** 293.

Cooper, W. C., and Gaffey, W. R. (1975). Mortality of lead workers. *J. Occup. Med.*, **17,** 100.

Cutler, B. S., Forbes, A. P., Ingersole, F. M., and Scully, R. E. (1972). Endometrial carcinoma after stilbestrol therapy in gonadal dysgenesis. *N. Engl. J. Med.*, **287,** 628.

Dales, L. G., Friedman, G. D., Ury, H. K., Grossman, S., and Williams, S. R. (1978). A case–control study of relationships of diet and other traits to colorectal cancer in American blacks. *Am. J. Epidemiol.*, **109,** 132.

Davies, I. J., Musa, M., and Dormandy, T. L. (1968). Measurements of plasma zinc. Part II. In malignant disease. *J. Clin. Pathol.*, **21,** 363.

DeWys, W., and Pories, W. (1972). Inhibition of a spectum of animal tumours by dietary zinc deficiency. *J. Natl. Cancer Inst.*, **48,** 375.

DeWys, W., Pories, W. J., Richter, M. C., and Strain, W. H. (1970). Inhibition of Walker 256 carcinosarcoma growth by dietary zinc deficiency. *Proc. Soc. Exp. Biol. Med.*, **135,** 17.

Dingwall-Fordyce, I., and Lane, R. E. (1963). A follow-up study of lead workers. *Br. J. Ind. Med.*, **20,** 313.

Doll, R., and Peto, R. (1981). The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.*, **66,** 1191.

Drasar, B. S., and Irving, D. (1973). Environmental factors and cancer of colon and breast. *Br. J. Cancer*, **27,** 167.

Duncan, J. R., and Dreosti, I. E. (1975). Zinc intake, neoplastic DNA synthesis, and chemical carcinogenesis in rats and mice. *J. Natl. Cancer Inst.*, **55,** 195.

Fenton, M. R., Burke, J. P., Tursi, F. D., and Arena, F. P. (1980). Effect of a zinc-deficient diet on the growth of an IgM-secreting plasmacytoma (TEPC-183). *J. Natl. Cancer Inst.*, **65,** 1271.

Fitzhugh, O. G. (1964). Appraisal of the safety of residues of veterinary drugs and their metabolites in edible animal tissues. *Ann. N.Y. Acad. Sci.*, **111,** 665.

Flavor and Fragrance Materials (1981). Allured Publishing Co., Wheaton, Illinois.

Fleiszer, D. M., Murray, D., Richards, G. K., and Brown, R. A. (1980). Effects of diet on chemically induced bowel cancer. *Can. J. Surg.*, **23,** 67.

Fong, L. Y. Y., Sivak, A., and Newberne, P. M. (1978). Zinc deficiency and methylbenzylnitrosamine-induced esophageal cancer in rats. *J. Natl. Cancer Inst.*, **61,** 145.

Freeman, H. J., Spiller, G. A., and Kim, Y. S. (1980). A double-blind study on the effects of differing purified cellulose and pectin fiber diets on 1,2-dimethylhydrazine-induced rat colonic neoplasia. *Cancer Res.*, **40,** 2661.

Furst, A. (1979). Problems in metal carcinogenesis. In Kharasch, N. (Ed.) *Trace Metals in Health and Disease*, p. 83. Raven Press, New York.

Gabrial, G., Schrager, T., and Newberne, P. M. (1982). Zinc deficiency, alcohol and a retinoid: association with esophageal cancer in rats. *J. Natl. Cancer Inst.*, **168,** 785.

Galy, P., Touraine, R., Brune, J., Gallois, P., Roudier, P., Loire, R., Lheureux, P., and Wiesendanger, T. (1963). Les cancers broncho-pulmonaires de l'intoxication arsenicale chronique chez les viticulteurs du Beaujolais. *Lyon Med.*, **210,** 735.

Gaskill, S. P., McGuire, W. L., Osborne, C. K., and Stern, M. P. (1979). Breast cancer mortality and diet in the United States. *Cancer Res.*, **39,** 3628.

Gass, G. H., Brown, J., and Okey, A. B. (1974). Carcinogenic effects of oral diethylstilboestrol on C_3H mice with and without the mammary tumour virus. *J. Natl. Cancer Inst.*, **53,** 1369.

Gershoff, S. N., and McGandy, R. B. (1981). The effects of vitamin A-deficient diets containing lactose in producing bladder calculi and tumours in rats. *Am. J. Clin. Nutr.*, **34,** 483.

Glatt, H. R., Metzler, F., and Oesch, F. (1979). Diethylstilbestrol and 11 derivatives. A mutagenicity study with *S. typhimurium*. *Mutat. Res.*, **67,** 113.

Graham, S., Schotz, W., and Martino, P. (1972). Alimentary factors in the epidemiology of gastric cancer. *Cancer*, **30,** 927.

Gray, G. E., Pike, M. C., and Henderson, B. E. (1979). Breast-cancer incidence and mortality rates in different countries in relation to known risk factors and dietary practices. *Br. J. Cancer*, **39,** 1.

Greeder, G. A., and Milner, J. A. (1980). Factors influencing the inhibitory effect of selenium on mice inoculated with Ehrlich ascites tumour cells. *Science*, **209,** 825.

Greenwald, P. C., Nasca, P. C., Burnett, W. S., and Polan, A. (1973). Prenatal stilbestrol experience in mothers of young cancer patients. *Cancer*, **31,** 568.

Gregor, A., Lee, P. N., Roe, F. J. C., Wilson, M. J., and Melton, A. (1980). Comparison of dietary histories in lung cancer cases and controls with special reference to vitamin A. *Nutr. Cancer*, **2,** 93.

Gregor, O., Toman, R., and Prusova, F. (1969). Gastrointestinal cancer and nutrition. *Gut*, **10,** 1031.

Haber, S., and Wissler, R. (1962). Effect of vitamin E on carcinogenicity of MCA. *Proc. Soc. Exp. Biol. Med.*, **111,** 774.

Hass, G. M., McDonald, J. H., Oyasu, R., Battifora, H. A., and Paloucek, J. T. (1967). Renal neoplasia induced by combinations of dietary lead subacetate and N-2-fluorenylacetamide. In King, J. S. (Ed.) *Renal Neoplasia*, p. 377. Little, Brown, Boston.

Hems, G. (1978). The contributions of diet and childbearing to breast-cancer rates. *Br. J. Cancer*, **37,** 974.

Hems, G. (1980). Associations between breast-cancer mortality rates, childbearing and diet in the United Kingdom. *Br. J. Cancer*, **41,** 429.

Herbst, A. L., and Cole, P. (1978). Epidemiologic and clinical aspects of clear cell adenocarcinoma in young women. In Herbst, A. L. (Ed.) *Intrauterine Exposure to Diethylstilbestrol in the Human*, pp. 2–7. American College of Obstetrics and Gynecology, Chicago, Illinois.

Hicks, R. M., Chowaniec, J., and Wakefield, J. St. J. (1978). Experimental induction of bladder tumours by a two-stage system. In Slaga, T. J., Sivak, A., and Boutwell, R. K. (Eds.) *Carcinogenesis—A Comprehensive Survey, Vol. 2, Mechanisms of Tumour Promotion and Cocarcinogenesis*, p. 475. Raven Press, New York.

Hill, M., MacLennan, R., and Newcombe, K. (1979). Letter to the editor. Diet and large-bowel cancer in three socioeconomic groups in Hong Kong. *Lancet*, **i,** 436.

Hollstein, M., McCann, J., Angelosanto, F. A., and Nichols, W. W. (1979). Short-term tests for carcinogens and mutagens. *Mutat. Res.*, **65,** 133.

Homburger, F. (1978). Negative lifetime carcinogen studies in rats and mice fed 50,000 ppm saccharin. In Galli, C. L., Paoletti, R., and Vettorazzi, G. (Eds.) *Chemical Toxicology of Food*, p. 359. Elsevier/North-Holland Biomedical Press, New York.

Hoover, R. N., and Strasser, P. H. (1980). Editorial. Saccharin. A bitter aftertaste? *N. Engl. J. Med.*, **302,** 573.

Hueper, W. C., and Payne, W. W. (1962). Experimental studies in metal carcinogenesis: chromium, nickel, iron, arsenic. *Arch. Environ. Health*, **5,** 445.

Huggins, C., and Yang, N. C. (1962). Induction and extinction of mammary cancer. *Science*, **137,** 257.

IARC (1974). *Some Organochlorine Pesticides*. IARC Monograph, Vol. 5. International Agency for Research on Cancer, Lyon, France: 241 pages.

IARC (1978). *Polychlorinated Biphenyls and Polybrominated Biphenyls*. IARC Monograph, Vol. 18, p. 37. International Agency for Research on Cancer, Lyon, France.

IARC (1980). *Some Metals and Metallic Compounds*. IARC Monograph, Vol. 23, pp. 101–112. International Agency for Research on Cancer, Lyon, France.

Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Fishbein, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., and Gart, J. J. (1969). Bioassay of pesticides and industrial chemicals for tumourigenicity in mice: a preliminary note. *J. Natl. Cancer Inst.*, **42,** 1101.

Ip, C., and Sinha, D. K. (1981). Enhancement of mammary tumorigenesis by dietary selenium deficiency in rats with a high polyunsaturated fat intake. *Cancer Res.*, **41,** 31.

Ishii, H., Koshimizu, T., Usami, S., and Fujimoto, T. (1981). Toxicity of aspartame and its diketopiperazine for Wistar rats by dietary administration for 104 weeks. *Toxicology*, **21,** 91.

Ishii, K., Nakamura, K., Ozaki, H., Yamada, N., and Takeuchi, T. (1968). Epidemiological problems of pancreas cancer. *Jpn. J. Clin. Med.*, **26,** 1839 (in Japanese).

Ito, N. (1973). Experimental studies on tumours of the urinary system of rats induced by chemical carcinogens. *Acta Pathol. Jpn.*, **23,** 87.

Ito, N., Hiasa, Y., Kamamoto, Y., Makiura, S., Sugihara, S., Marugami, M., and Okajima, E. (1971). Histopathological analysis of kidney tumours in rats induced by chemical carcinogens. *Gann*, **62,** 435.

Ivett, J. L., and Tice, R. R. (1981). Diethylstilbestrol-diphosphate induces chromosomal aberrations but not SCE in murine bone marrow cells *in vivo*. *Environ. Mutagen.*, **3,** 445.

Jacobs, M. M., Forst, C. F., and Beams, F. A. (1981). Biochemical and clinical effects of selenium on DMH-induced colon cancer in rats. *Cancer Res.*, **41,** 4458.

Jain, M., Cook, G. M., Davis, F. G., Grace, M. G., Howe, G. R., and Miller, A. B. (1980). A case–control study of diet and colorectal cancer. *Int. J. Cancer*, **26,** 757.

Jelinek, C. F. (1981). Occurrence and methods of control of chemical contaminants in foods. *Environ. Health Perspect.*, **39,** 143.

Kang, H. K., Infante, P. F., and Carra, J. S. (1980). Occupational lead exposure and cancer. *Science*, **207,** 935.

Kay, K. (1977). Polybrominated biphenyls (PBBs). Environmental contaminants in Michigan 1973–1976. *Environ. Res.*, **13,** 74.

Kimbrough, R. D., and Linder, R. E. (1974). Induction of adenofibrosis and hepatomas of the liver in BALB/cJ mice by polychlorinated biphenyls (Aroclor 1254). *J. Natl. Cancer. Inst.*, **53,** 547.

Kimbrough, R. D., Burse, V. W., and Liddle, J. A. (1978). Persistent liver lesions in rats after a single dose of PBBs (Firemaster FF-1) and concomitant PBB tissue levels. *Environ. Health Perspect.*, **23,** 265.

Kimbrough, R. D., Groce, D. F., Korner, M. P., and Burse, V. W. (1981). Induction of liver tumours in female Sherman strain of rats by polybrominated biphenyls. *J. Natl. Cancer Inst.*, **66,** 535.

Kimura, N. T., Kanematsu, T., and Baba, T. (1976). Polychlorinated biphenyls as a promoter in hepatocarcinogenesis. *Z. Krebs Forsch. Klin. Onkol.*, **87,** 257.

Kipling, M. D., and Waterhouse, J. A. H. (1967). Letter to the editor. Cadmium and prostatic carcinoma. *Lancet*, **i,** 730.

Klein, M. (1963). Susceptibility of strain B6AF$_1$/J hybrid infant mice to tumorigenesis with 1,2-benzanthracene, deoxycholic acid, and 3-methylcholanthrene. *Cancer Res.*, **23,** 1701.

Kolonel, L. N. (1976). Association of cadmium with renal cancer. *Cancer*, **37,** 1782.

Kolonel, L. N., and Winkelstein, W. (1977). Cadmium and prostatic carcinoma. *Lancet*, **ii,** 566.

Kolonel, L. N., Hankin, J. H., Lee, J., Chu, S. Y., Nomura, A. M. Y., and Hinds, M. W. (1981). Nutrient intakes in relation to cancer incidence in Hawaii. *Br. J. Cancer*, **44,** 332.

Kroes, R., Peters, W. J., Berkvens, J., Verschuaren, G., deVries, T., and VanEsch, G. (1977). Long-term toxicity and reproduction study with cyclamate, saccharin and cyclohexylamine. *Toxicology*, **8,** 285.

Kuratsune, M. (1976). Epidemiologic studies on Yusho. In Higuchi, K. (Ed.) *PCB Poisoning and Pollution*, p. 9. Kodansha Ltd., Tokyo. Academic Press, New York.

Kuratsune, M., Masuda, Y., and Nagayama, J. (1976). Some of the recent findings concerning Yusho. In *National Conference on Polychlorinated Biphenyls, Chicago, Illinois, November 1975*, p. 14. EPA-560/6-75-004. Office of Toxic Substances, US Environmental Protection Agency, Washington, DC.

Kutz, F., and Strassman, S. C. (1976). *PCBs in US Populations*. US Environmental Protection Agency, Washington, DC.

Kwan, K. W. (1937). Carcinoma of the esophagus. A statistical study. *Clin. Med. J. (Peking)*, **52,** 237.

Latarjet, M., Galy, P., Maret, G., and Gallois, P. (1964). Cancers broncho-pulmonaires et intoxication arsenicale chez des vignerons du Beaujolais. *Mem. Acad. Chir.*, **90,** 384.

Lemen, R. A., Lee, J. S., Wagoner, J. K., and Blejer, H. P. (1976). Cancer mortality among cadmium production workers. *Ann. N.Y. Acad. Sci.*, **271,** 273.

Lew, E. A., and Garfinkel, L. (1979). Variations in mortality by weight among 750,000 men and women. *J. Chron. Dis.*, **32,** 563.

Lin, H. J., Chan, W. C., Fong, Y. Y., and Newberne, P. M. (1977). Zinc levels in serum, hair and tumours from patients with esophageal cancer. *Nutr. Rep. Int.*, **15,** 635.

Liu, K., Stamler, J., Moss, D., Garside, D., Persky, V., and Soltero, I. (1979). Dietary cholesterol, fat, and fibre, and colon-cancer mortality. *Lancet*, **ii,** 782.

Locniskar, M., Nauss, K., and Newberne, P. M. (1983). The effect of quality and quantity of dietary fat on the immune system. *J. Nutr.*, **113,** 951.

Lubin, J. H., Burns, P. E., Blot, W. J., Ziegler, R. G., Lees, A. W., and Fraumeni, J. F., Jr. (1981). Dietary factors and breast cancer risk. *Int. J. Cancer*, **28,** 685.

MacLennan, R., DaCosta, J., Day, N. E., Law, C. H., Ng, Y. K., and Shanmugaratnam, K. (1977). Risk factors for lung cancer in Singapore Chinese, a population with high female incidence rates. *Int. J. Cancer*, **20,** 854.

MacLennan, R., Jensen, O. M., Mosbech, J., and Vuori, H. (1978). Diet, transit time, stool weight, and colon cancer in two Scandinavian populations. *Am. J. Clin. Nutr.*, **31,** S239.

Madhavan, T. V., and Gopalan, C. (1968). The effect of dietary protein on carcinogenesis of aflatoxin. *Arch. Pathol.*, **85,** 133.

Mao, P., and Molnar, J. J. (1967). The fine structure and histochemistry of lead-induced renal tumours in rats. *Am. J. Pathol.*, **5,** 571.

Martin, C. N., McDermid, A. C., and Garner, R. C. (1978). Testing of known carcinogens and non-carcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells. *Cancer Res.*, **38,** 2621.

Martinez, I., Torres, R., Frias, Z., Colon, J. R., and Fernandez, M. (1979). Factors

associated with adenocarcinomas of the large bowel in Puerto Rico. In Birch, J. M. (Ed.) *Advances in Medical Oncology, Research and Education, Vol. 3, Epidemiology*, p. 45. Pergamon Press, Oxford.

Medina, D., Lane, H., and Tracy, C. M. (1983). Selenium and mouse mammary tumorigenesis: an investigation of possible mechanisms. *Cancer Res.*, **43,** 2460s.

Medina, D., and Shepherd, F. (1980). Selenium-mediated inhibition of mouse mammary tumorigenesis. *Cancer Lett.*, **8,** 241.

Mergens, W. J., Vane, F. M., Tannenbaum, S. R., Green, L., and Skipper, P. L. (1979). *In vitro* nitrosation of methapyrilene. *J. Pharm. Sci.*, **68,** 827.

Mettlin, C., Graham, S., and Swanson, M. (1979). Vitamin A and lung cancer. *J. Natl. Cancer Inst.*, **62,** 1435.

Mettlin, C., Graham, S., Priore, R., Marshall, J., and Swanson, M. (1981). Diet and cancer of the esophagus. *Nutr. Cancer*, **2,** 143.

Mgbodile, M. U. K., and Campbell, T. C. (1972). Effect of protein deprivation of male weanling rats on the kinetics of hepatic microsomal enzyme activity. *J. Nutr.*, **102,** 53.

Miller, A. B., Kelly, A., Choi, N. W., Matthews, V., Morgan, R. W., Munan, L., Burch, J. D., Feather, J., How, G. R., and Jain, M. (1978). A study of diet and breast cancer. *Am. J. Epidemiol.*, **107,** 499.

Mirvish, S. S. (1981). Inhibition of the formation of carcinogenic *N*-nitroso compounds by ascorbic acid and other compounds. In Burchenal, J. H., and Oettgen, H. F. (Eds.) *Cancer: Achievements, Challenges, and Prospects for the 1980s*, Vol. 1, p. 557. Grune and Stratton, New York.

Mirvish, S. S. (1983). The etiology of gastric cancer. *J. Natl. Cancer Inst.*, **71,** 631.

Mirvish, S. S., Wallcave, L., Eagen, M., and Shubik, P. (1972). Ascorbate–nitrite reaction: possible means of blocking the formation of carcinogenic *N*-nitroso compounds. *Science*, **177,** 65.

Morgan, R. W., and Jain, M. G. (1974). Bladder cancer: smoking, beverages and artificial sweeteners. *Can. Med. Assoc. J.*, **111,** 1007.

Morrison, A. S., and Buring, J. E. (1980). Artificial sweeteners and cancer of the lower urinary tract. *N. Engl. J. Med.*, **302,** 537.

Moryeau, P., Bailone, A., and Devoret, R. (1976). Prophage induction in *Escherichia coli envA uvrB*: a highly sensitive test for potential carcinogens. *Proc. Natl. Acad. Sci. (USA)*, **73,** 3700.

Nagao, M., and Sugimura, T. (1978). Mutagenesis: microbial systems. In Gelboin, H. V., and Ts'o, P. O. P. (Eds.) *Polycyclic Hydrocarbons and Cancer, Vol. 2, Molecular and Cell Biology*, p. 99. Academic Press, New York.

NAS (1971). *Selenium in Nutrition*. Subcommittee on Selenium, Committee on Animal Nutrition, National Academy of Sciences, Washington, DC: 79 pages.

NAS (1978). *Saccharin: Tech-assess of Risks and Benefits*. National Academy of Sciences, Washington, DC: 250 pages.

NAS (1982). *Diet, Nutrition and Cancer*. National Academy of Sciences, Washington, DC.

NCI (1977). *Bioassay of Lindane for Possible Carcinogenicity*. NCI Technical Report Series No. 14. DHEW Publication No. (NIH) 77-814. National Cancer Institute, Bethesda, Maryland: 99 pages.

NCI (1978a). *Bioassay of Malathion for Possible Carcinogenicity*. NCI Technical Report Series No. 24. DHEW Publication No. (NIH) 78-824. National Cancer Institute, Bethesda, Maryland: 102 pages.

NCI (1978b). *Bioassays of Aldrin and Dieldrin for Possible Carcinogenicity*. NCI Technical Report Series No. 21. DHEW Publication No. (NIH) 78-821. National Cancer Institute, Bethesda, Maryland: 184 pages.

NCI (1979). *Bioassay of Butylated Hydroxytoluene (BHT) for Possible Carcinogenicity*. NCI Carcinogenesis Technical Report Series No. 150. NIH Publication No. 79-1706. Carcinogenesis Testing Programme, National Cancer Institute, Bethesda, Maryland: 114 pages.

Nelson, A. A., Fitzhugh, O. G., and Calvery, H. O. (1943). Liver tumours following cirrhosis caused by selenium in rats. *Cancer Res.*, **3,** 230.

Nelson, W. C., Lykins, M. H., Mackey, J., Newill, V. A., Finklea, J. F., and Hammer, D. I. (1973). Mortality among orchard workers exposed to lead arsenate spray: a cohort study. *J. Chron. Dis.* **26,** 105.

Newberne, P. M. (1974). Mycotoxins: toxicity, carcinogenicity and the influence of various nutritional conditions. *Environ. Health Perspect.*, **9,** 1.

Newberne, P. M., and Conner, M. (1974). Acute studies with selenium and aflatoxin B_1 liver injury. *Trace Substances Conference VIII, University of Missouri*, p. 323.

Newberne, P. M., and McConnell, R. G. (1980a). Dietary nutrients and contaminants in laboratory animal experimentation. *J. Environ. Pathol. Toxicol.*, **4,** 105.

Newberne, P. M., and McConnell, R. G. (1980b). Nutrient deficiencies in cancer causation. *J. Environ. Pathol. Toxicol.*, **3,** 323–356.

Newberne, P. M., Rogers, A. E., and Gross, R. L. (1977). Nutritional modulation of carcinogenesis. In Nieburgs, H. E. (Ed.) *Prevention and Detection of Cancer, Part I, Prevention, Vol. 1, Etiology*, pp. 665–691. Marcel Dekker, New York.

Newberne, P. M., Weigert, J., and Kula, N. (1979). Quality and quantity of dietary fat hepatic mixed function oxidases and hepatocellular carcinoma induced by aflatoxin B_1 in rats. *Cancer Res.*, **39,** 3986.

Newberne, P. M., Rogers, A. E., and Nauss, K. M. (1983). Choline, methionine and related factors in oncogenesis. In Butterworth, C. E., and Hutchinson, M. (Eds.) *Nutritional Factors in the Induction and Maintenance of Malignancy*, p. 247. Academic Press, New York.

Nomura, A., Henderson, B. E., and Lee, J. (1978). Breast cancer and diet among the Japanese in Hawaii. *Am. J. Clin. Nutr.*, **31,** 2020.

Nordensen, I., Beckman, G., Beckman, L., and Nordstrom, S. (1978). Occupational and environmental risks in and around a smelter in northern Sweden. II. Chromosomal aberrations in workers exposed to arsenic. *Hereditas*, **88,** 47.

Okuda, K., Nakashima, T., Sakamoto, K., Ikari, T., Hidaka, H., Kubo, Y., Sakuma, K., Motoike, Y., Okuda, H., and Obata, H. (1982). Hepatocellular carcinoma arising in non-cirrhotic and highly cirrhotic livers. A comparative study of histopathology and frequency of hepatitis B markers. *Cancer,* **49,** 450.

Peers, F. G., and Linsell, C. A. (1973). Dietary aflatoxins and liver cancer—a population based study in Kenya. *Br. J. Cancer*, **27,** 473.

Peirce, W. E. H. (1961). Tumour-promotion by lime oil in the mouse forestomach. *Nature*, **189,** 497.

Petering, H. G., Buskirk, H. H., and Crim, J. A. (1967). The effect of dietary mineral supplements of the rat on the antitumour activity of 3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazone). *Cancer Res.*, **27,** 1115.

Petres, J., Baron, D., and Hagedorn, M. (1977). Effects of arsenic cell metabolism and cell proliferation: cytogenetic and biochemical studies. *Environ. Health Perspect.*, **19,** 223.

Petres, J., Schmid-Ullrich, K., and Wolf, U. (1970). Chromosomen-aberrationen an menschlichen lymphozyten bei chronischen arsenschaden. *Dtsch. Med. Wochenschr.*, **95,** 79.

Phillips, R. L. (1975). Role of lifestyle in dietary habits in risk of cancer among Seventh-Day Adventists. *Cancer Res.*, **35,** 3513.

Phillips, R. L., Kuzma, J. W., Bisson, W. L., and Lotz, T. (1980). Influence of selection versus lifestyle on risk of fatal cancer and cardiovascular disease among Seventh-Day Adventists. *Am. J. Epidemiol.*, **112,** 296.

Poswillo, D. E., and Cohen, B. (1971). Inhibition of carcinogenesis by dietary zinc. *Nature*, **231,** 447.

Prasad, A. S. (Ed.) (1978). *Trace Elements and Iron in Human Metabolism*. Plenum Publishing Corp., New York.

Preston, B. D., Van Miller, J. P., Moore, R. W., and Allen, J. R. (1981). Promoting effects of polychlorinated biphenyls (Aroclor 1254) and polychlorinated dibenzofuran-free Aroclor 1254 on diethylnitrosamine-induced tumorigenesis in the rat. *J. Natl. Cancer Inst.*, **66,** 509.

Preston, R. S., Hayes, J. R., and Campbell, T. C. (1976). The effect of protein deficiency on the *in vivo* binding of aflatoxin B_1 to rat liver macromolecules. *Life Sci.*, **19,** 1191.

Rigdon, R. H., and Neal, J. (1969). Relationship of leukemia to lung and stomach tumours in mice fed benzo[*a*]pyrene. *Proc. Soc. Exp. Biol. Med.*, **130,** 146.

Robinson, T. R. (1976). The health of long service tetraethyl lead workers. *J. Occup. Med.*, **18,** 31.

Roe, F. J. C., Levy, L. S., and Carter, R. L. (1970). Feeding studies on sodium cyclamate, saccharin and sucrose for carcinogenic and tumour-promoting activity. *Food Cosmet. Toxicol.*, **8,** 135.

Rogers, A. E., and Newberne, P. M. (1980). Lipotrope deficiency in experimental carcinogenesis. *Nutr. Cancer*, **2,** 104.

Rogers, A. E., and Wetsel, W. C. (1981). Mammary carcinogenesis in rats fed different amounts and types of fat. *Cancer Res.*, **41,** 3735.

Rosenkranz, H. S., and Poirier, L. A. (1979). Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and non-carcinogens in microbial systems. *J. Natl. Cancer Inst.*, **62,** 873.

Roth, F. (1957). Arsen–Leber–Tumoren (Hamangioendotheliom). *Z. Krebsforsch.*, **61,** 468.

Rustia, M. (1979). Role of hormone imbalance in transplacental carcinogenesis induced in Syrian golden hamsters by sex hormones. *Natl. Cancer Inst. Monogr.*, **51,** 77.

Schrauzer, G. N. (1976). Selenium and cancer: a review. *Bioinorg. Chem.*, **5,** 275.

Schrauzer, G. N. (1979). Trace elements in carcinogenesis. In Draper, H. H. (Ed.) *Advances in Nutritional Research*, Vol. 2, p. 219. Plenum Publishing Corp., New York.

Schrauzer, G. N., White, D. A., and Schneider, C. J. (1977a). Cancer mortality correlation studies. III. Statistical associations with dietary selenium intakes. *Bioinorg. Chem.*, **7,** 23.

Schrauzer, G. N., White, D. A., and Schneider, C. J. (1977b). Cancer mortality correlation studies. IV. Associations with dietary intakes and blood levels of certain trace elements, notably Se-antagonists. *Bioinorg. Chem.*, **7,** 35.

Schrauzer, G. N., White, D. A., and Schneider, C. J. (1978). Selenium and cancer: effects of selenium and of the diet on the genesis of spontaneous mammary tumours in virgin inbred female C_3H/St mice. *Bioinorg. Chem.*, **8,** 387.

Schroeder, H. A., Balassa, J. J., and Vinton, W. H., Jr. (1964). Chromium, lead, cadmium, nickel and titanium in mice: effect on mortality, tumours and tissue levels. *J. Nutr.*, **83,** 239.

Schroeder, H. A., Balassa, J. J., and Vinton, W. H., Jr. (1965). Chromium, cadmium and lead in rats: effects on lifespan, tumours and tissue levels. *J. Nutr.*, **86,** 51.

Searle, G. D., & Co. (1974a). *104-Week Toxicity Study in the Mouse*. P-T No. 984H73. Final Report. G. D. Searle & Co., Skokie, Illinois: 295 pages.

Searle, G. D., & Co. (1974b). *An Evaluation of Mutagenic Potential Employing the Host-*

mediated Assay in the Mouse. P-T No. 1095S73. G. D. Searle & Co., Skokie, Illinois: 23 pages.

Shakerin, M., and Paloucek, J. (1965). Intranuclear inclusions and renal tumours in rats fed lead subacetate. *Lab. Invest.*, **14,** 592.

Shamberger, R. J. (1970). Relation of selenium to cancer. I. Inhibitory effect of selenium on carcinogenesis. *J. Natl. Cancer Inst.*, **44,** 931.

Shamberger, R. J. (1971). Inhibitory effects of vitamin A on carcinogenesis. *J. Natl. Cancer Inst.*, **47,** 667.

Shamberger, R. J., and Willis, C. E. (1971). Selenium distribution and human cancer mortality. *CRC Crit. Rev. Clin. Lab. Sci.*, **2,** 211.

Shamberger, R. J., Rukovena, E., Longfield, A. K., Tytko, S. A., Deodhar, S., and Willis, C. E. (1973). Antioxidants and cancer. I. Selenium in the blood of normal and cancer patients. *J. Natl. Cancer Inst.*, **50,** 863.

Shamberger, R. J., Tytko, S. A., and Willis, C. E. (1976). Antioxidants and cancer. Part VI. Selenium and age-adjusted human cancer mortality. *Arch. Environ. Health*, **31,** 231.

Shank, R. C. (1981). Mycotoxins and *N*-nitroso compounds: environmental risks. *CRC Rev.*, **1,** 227.

Shekelle, R. B., Liu, S., Raynor, W. J., Jr., Lepper, M., Maliza, C., and Rossof, A. H. (1981). Dietary vitamin A and risk of cancer in the Western Electric Study. *Lancet*, **ii,** 1185.

Shiraishi, Y., Kurahashi, H., and Yosida, T. H. (1972). Chromosomal aberrations in cultured human leukocytes induced by cadmium sulfide. *Proc. Jpn. Acad.*, **48,** 133.

Shklar, G. (1982). Oral mucosal carcinogenesis in hamsters: inhibition by vitamin E. *J. Natl. Cancer Inst.*, **68,** 791.

Simmon, V. F., Rosenkranz, H. S., Zeiger, E., and Poirier, L. A. (1979). Mutagenic activity of chemical carcinogens. *J. Natl. Cancer Inst.*, **62,** 911.

Sirover, M. A., and Loeb, L. A. (1976). Infidelity of DNA synthesis *in vitro*: screening for potential metal mutagens or carcinogens. *Science*, **194,** 1434.

Smith, P. G., and Jick, H. (1978). Cancers among users of preparations containing vitamin A. *Cancer*, **42,** 808.

Soloway, M., Cohen, S. M., Dekernion, J. B., and Perske, J. (1975). Failure of ascorbic acid to inhibit FANFT-induced bladder cancer. *J. Urol.*, **113,** 483.

Sporn, M. B., and Newton, D. L. (1979). Chemoprevention of cancer with retinoids. *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **38,** 2528.

Sporn, M. B., and Newton, D. L. (1981). Recent advances in the use of retinoids for cancer prevention. In Burchenal, J. H., and Oettgen, H. F. (Eds.) *Cancer: Achievements, Challenges, and Prospects for the 1980s*, Vol. 1, p. 541. Grune and Stratton, New York.

Sporn, M. B., Dunlop, N. M., Newton, D. L., and Smith, J. M. (1976). Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **35,** 1332.

Stemmerman, G., Haenzel, W., and Locke, F. (1977). Epidemiologic pathology of gastric ulcer and carcinoma in Japanese. *J. Natl. Cancer Inst.*, **58,** 13.

Strain, W. H., Mansour, E. G., Flynn, A., Pories, W. J., Tomaro, A. J., and Hill, O. A., Jr. (1972). Letter to the editor. Plasma-zinc concentration in patients with bronchogenic cancer. *Lancet*, **i,** 1021.

Suphakarn, V. S., Newberne, P. M., and Goldman, M. (1983). Vitamin A and aflatoxin: effect on liver and colon cancer. *Nutr. Cancer*, **5,** 41–50.

Tannenbaum, A. (1942a). The genesis and growth of tumours. II. Effects of caloric restriction *per se*. *Cancer Res.*, **2,** 460.

Tannenbaum, A. (1942b). The genesis and growth of tumours. III. Effects of a high-fat diet. *Cancer Res.*, **2,** 468.

Tannenbaum, A. (1944). The dependence of the genesis of induced skin tumours on the caloric intake during different stages of carcinogenesis. *Cancer Res.*, **4,** 673.

Tannenbaum, A. (1945a). The dependence of tumour formation on the degree of caloric restriction. *Cancer Res.*, **5,** 609.

Tannenbaum, A. (1945b). The dependence of tumour formation on the composition of the calorie-restricted diet as well as on the degree of restriction. *Cancer Res.*, **5,** 616.

Temcharoen, P., Anukarahanonta, T., and Bhamarapravati, N. (1978). Influence of dietary protein and vitamin B_{12} on the toxicity and carcinogenicity of aflatoxins in rat liver. *Cancer Res.*, **38,** 2185.

Teodorescu, F., and Calugaru, A. (1972). Modificari chromozomiale in celulele etc., *Stud. Cercet. Biol. Ser. Zool.*, **24,** 451.

Thompson, H. J., and Becci, P. J. (1980). Selenium inhibition of *N*-methyl-*N*-nitrosourea-induced mammary carcinogenesis in the rat. *J. Natl. Cancer Inst.*, **65,** 1299.

Thorpe, E., and Walker, A. (1973). Toxicology of dieldrin II. *Food Cosmet. Toxicol.*, **11,** 433.

Tong, C., Laspia, M. F., Telang, S., and Williams, G. M. (1981). The use of adult rat liver cultures in detection of genotoxicity of various polycyclic aromatic hydrocarbons. *Environ. Mutagen.* **3,** 477.

Trosko, J. E., Yotti, L. P., Warren, S., Tsushimoto, G., and Chang, C. C. (1982). Inhibition of cell–cell communication by tumour promoters. In Hecker, E., Kunz, W., Marx, S., Fusenig, N. E., and Phielman, H. W. (Eds.) *Carcinogenesis: A Comprehensive Survey, Vol. 7, Carcinogenesis and Biological Effects of Tumour Promoters*, pp. 565–585. Raven Press, New York.

Tseng. W. P. (1977). Effects and dose–response relationships of skin cancer and blackfoot disease with arsenic. *Environ. Health Perspect.*, **19,** 109.

Tseng, W. P., Chu, H. M., How, S. W., Fong, J. M., Lin, C. S., and Yeh, S. (1968). Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. *J. Natl. Cancer Inst.*, **40,** 453.

Tsuchiya, K. (1977). Various effects of arsenic in Japan. *Environ. Health Perspect.*, **19,** 35.

USDA (US Department of Agriculture) (1978). Situation and outlook. *Sugar Sweetener Rep.* **3(5),** 1–70.

USEPA (1975). *Scientific and Technical Assessment Report on Particulate Polycyclic Organic Matter (PPOM)*. EPA-600/6-75-001. Office of Research and Development, US Environmental Protection Agency, Washington, DC: 95 pages.

USEPA (1979). Polychlorinated biphenyls (PCBs). Manufacturing, processing, distribution in commerce, and use prohibitions. *Fed. Regist.*, **44,** 31514.

USEPA (1980). *Summary of Reported Incidents Involving Toxaphene*. Pesticide Incident Monitoring System Report No. 316. Hazard Evaluation Division, Office of Pesticide Programs, US Environmental Protection Agency, Washington, DC.

USFDA (1977). Polychlorinated biphenyls (PCBs). Unavoidable contaminants in food and food packaging materials; reduction of temporary tolerances. *Fed. Regist.*, **42,** 17487.

VanEsch, G. J., and Kroes, R. (1969). The induction of renal tumours by feeding basic lead acetate to mice and hamsters. *Br. J. Cancer*, **23,** 765.

VanEsch, G. J., VanGenderen, H., and Vink, H. H. (1962). The incidence of renal tumours by feeding of basic lead acetate to rats. *Br. J. Cancer*, **16,** 289.

Voegtlin, C., and Maver, M. E. (1936). Lysine and malignant growth. II. The effect on malignant growth of a gliadin diet. *Public Health Rep.*, **51,** 1436.

Voegtlin, C., and Thompson, J. W. (1936). Lysine and malignant growth. I. The amino acid lysine as a factor controlling the growth rate of a typical neoplasm. *Public Health Rep.*, **51,** 1429.

Volgarev, M. N., and Tscherkes, L. A. (1967). Further studies in tissue changes associated with sodium selenate. In *Selenium in Biomedicine, First International Symposium*, p. 179. AVI Publishing Co., Westport, Connecticut.

Wald, N., Idle, M., Boreham, J., and Bailey, A. (1980). Low serum vitamin A and subsequent risk of cancer: preliminary results of a prospective study. *Lancet*, **ii,** 813.

Wang, H. H., and Grufferman, S. (1981). Aplastic anemia and occupational pesticide exposure: a case–control study. *J. Occup. Med.*, **23,** 364.

Wang, H. H., and MacMahon, B. (1979). Mortality of workers employed in the manufacture of chlordane and heptachlor. *J. Occup. Med.*, **21,** 745.

Wassertheil-Smoller, S., Romney, S. L., Wylie-Rosett, J., Slagle, S., Miller, G., Lucido, D., Duttagupta, C., and Palan, P. R. (1981). Dietary vitamin C and uterine cervical dysplasia. *Am. J. Epidemiol.*, **114,** 714.

Watanabe, K., Reddy, B. S., Wong, C. Q., and Weisburger, J. H. (1978). Effect of dietary undegraded carrageenin on colon carcinogenesis in F344 rats treated with azoxymethane or methylnitrosourea. *Cancer Res.*, **38,** 4427.

Watanabe, K., Reddy, B. S., Weisburger, J. H., and Kritchevsky, D. (1979). Effect of dietary alfalfa, pectin, and wheat bran on azoxymethane- or methylnitrosourea-induced colon carcinogenesis in F344 rats. *J. Natl. Cancer Inst.*, **63,** 141.

Wattenberg, L. W. (1972). Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. *J. Natl. Cancer Inst.*, **48,** 1425.

Wells, P., Alftergood, L., and Alfin-Slater, R. B. (1976). Effect of varying levels of dietary protein on tumour development and lipid metabolism in rats exposed to aflatoxin. *J. Am. Oil Chem. Soc.*, **53,** 559.

Wetsel, W. C., Rogers, A. E., and Newberne, P. M. (1981). Dietary fat and DMBA mammary carcinogenesis. *Cancer Detect. Prev.*, **4,** 535.

Wiegand, R. G. (1978). Practical considerations for synthetic sweeteners: past, present and future—cyclamates. In Shaw, J. H., and Roussos, G. G. (Eds.) *Sweeteners and Dental Caries*, p. 263. Information Retrieval, Inc., Arlington, Virginia.

Wilson, R. B., Hutcheson, D. P., and Wideman, L. (1977). Dimethylhydrazine-induced colon tumours in rats fed diets containing beef fat or corn oil with and without wheat bran. *Am. J. Clin. Nutr.*, **30,** 176.

Witschi, H. P. (1981). Enhancement of tumour formation in mouse lung by dietary butylated hydroxytoluene. *Toxicology*, **21,** 95.

Yagi, T., and Nishioka, H. (1977). DNA damage and its degradation by metal compounds. *Sci. Eng. Rev. Doshisha Univ.*, **18,** 63.