SELECTED FOOD TOXICANTS

National Council for Scientific Research and Development,
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by

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Kuala Lumpur

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Ministry of Science, Technology and the Environment, Malaysia.
1984

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PREFACE

The study of toxicants in foods is a relatively unexplored field of research in Malaysia. Amongst the earliest toxicants studied were the aflatoxins. Local investigators directed their attention to these mycotoxins soon after their discovery in the 1960's. Of late, there appears to be some renewed interest in these toxicants. More recently other food toxicants were also investigated. These include lead and mercury, some antinutritional factors in foods (such as lectins, protease inhibitors, phytates, and tannins), nitrates and nitrites, and the cyclopropene fatty acids. The toxicological aspects of some food preservatives and additives have also drawn the attention of a few investigators. However, these studies are few and far in between, thus leaving a large void in our knowledge of the possible toxicants in Malaysian foods.

It can be envisaged that this field of research will attract more investigators in the years to come. With increasing sophistication, the consumer would be more demanding towards the safety of the foods he consumes. It is thus an area which cannot be neglected much further. The objective of this publication is therefore to provide the intending investigator with a fairly comprehensive review of some selected toxicants, those felt to be most relevant and pressing for attention. Due to limited knowledge and experience in this field, we have restricted ourselves to examining three food toxicants, namely aflatoxins, lead and the nitroso compounds. All efforts have however been made to include recent publications, and wherever possible, local studies on these toxicants. It is hoped that these reviews would contribute towards our understanding and knowledge of these toxicants.

We are grateful to Dr. Y.H. Chong, Head of the Division of Human Nutrition, Institute for Medical Research, and Dr. T.W. Lim, Director of the Institute, for their permission to publish these reviews. Our sincere thanks also go to Dr. A. Zaharudin Idrus of the National Science Council (MPKSN) for his encouragement and the Medical Sciences Committee of the MPKSN for their support.

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1 AFLATOXINS

1.1 Introduction

Fungal toxins are a group of important naturally occuring toxicants in foods. Over 45 mycotoxins are now known to be either carcinogenic and/or mutagenic (Stolof, 1981), most of which have been discovered only in the last 25 years or so. Aside from aflatoxin, which will be discussed in some detail in this chapter, other mycotoxins of importance are sterigmatocystin, ochratoxin A, zearalenone, T-2 toxin, patulin, penicillic acid, griseofulvin, luteoskyrin and cyclochlorotine. Besides sterigmatocystin, which is a very close structural relative of aflatoxin, the carcinogenic and mutagenic activities of these compounds have not been very consistent (Campbell, 1982). Furthermore, these has been no human epidemiological data on the cancer-producing potential of any of these compunds.

The best known of these fungal toxins or mycotoxins, is aflatoxin, although it was not the first to have been recognized. Unknown prior to the 60's, aflatoxin research today is world wide and is carried out by various disciplines, including food technology and agriculture, veterinary and human medicine and fundamental biochemistry. Originally discovered as a veterinary problem, involving principally turkeys, today aflatoxin has caused great concern as a possible health hazard to humans. A vast amount of literature has accumulated on the subject; numerous books have been written and available information constantly up-dated in various reviews. Recent comprehensive reviews are contained in two volumes edited by R.C. Shank (1981). In Malaysia, studies into the aflatoxin problem were carried out as early as in the 1960's. Early reviews on the subject include those of Moir and Varghese (1965) and Moir (1967). More recently, Abidin Hamid (1982) and Arokiasamy (1983) have in their reviews re-emphasized the dangers of aflatoxin contamination. This chapter will highlight some of the findings, including recent developments on this important mycotoxin with appropriate reference to research in Malaysia.

Aflatoxin is the generic term of a series of metabolites produced by the common yellow mould *Aspergillus flavus*. The most common member of this family of complex lactones is aflatoxin B1; it has been extensively and intensively studied. This compound has served as a popular example of risk assessment modeling, as a probe into the molecular genetics of chemical carcinogenesis, as a good example of the impact of nutrition on experimental carcinogenesis, as a probe into the role of carcinogen metabolism in carcinogenesis initiation, and as an illustration of selected characteristics of chemical carcinogen regulation (Campbell, 1982).

Aflatoxin is certainly one of the most potent chemical carcinogen known to man, producing liver tumours in rats fed with 1 μg per day, and local sarcomas in mice after subcutaneous injection of 10 μg (Butler, Greenblat and Lijinsky, 1969). Owing to its high toxicity and the broad range of animal species in which it is active, the relatively long-standing consensus of researchers in this field has always been that aflatoxin causes liver cancer in man. Epidemiological studies, particularly in several tropical areas of the world, have been reported to support this belief. Based on all available data, the International Agency for Research on Cancer (IARC) listed aflatoxin B1 as one of the 26 chemicals or industrial processes associated with or are strongly suspected to be associated with the occurrence of human cancer (Tomatis *et al,* 1978).

Since the aflatoxins have been found to be harmful to a wide variety of animal life and to isolated human cells and creating problems of health as well as social and economic significance, it is clear that all efforts should be made to reduce the level of this mycotoxin in our foods, if not to eliminate them entirely. The laws regulating the sale of foods in many countries have already included provisions aimed at controlling aflatoxins content in foods.

1.2 Discovery of The Aflatoxins

In 1960, more than 100,000 young turkeys on poultry farms in the south and east of England died in the course of a few months from an apparently new disease that was termed Turkey 'X' disease (Blount, 1961). Affected birds died within a week, during which they lost their appetitie, became markedly lethargic and developed a weakness of the wings. Post mortem examination showed haemorrhages or pale necrotic lesions in the liver and, frequently engorged kidneys.

It was soon found that the disease affected not only turkeys. Ducklings and young pheasants were also affected and heavy mortality was experienced. Reports were received from Kenya and Uganda of severe losses of ducklings from a similar disease (Asplin and Carnaghan, 1961). Almost simultaneously, in the United States an outbreak of trout hepatoma was discovered in the spring of 1960 after a shipment of trout raised in a commercial hatchery was inspected at the California state border and many of the fish were found to have hepatomas (Wolf and Jackson, 1963).

The disease caught the attention of many investigators who embarked immediately to determine its etiology. There was then no known relationship or common denominator between the various outbreaks reported. Veterinary examinations for pathogenic microorganisms were generally negative and attempts to demonstrate virus infection were unsuccessful. Turkey 'X' disease was soon shown to be dietary in origin and that a toxin was involved. It was discovered that a common ingredient of the feed given to affected turkeys from different parts of London was a Brazilian peanut meal (Blount, 1961).

It was found that this peanut meal was highly toxic to poults and ducklings with symptoms typical of turkey 'X' disease.

Attention was then focussed on the possibility that the toxin in the peanut meal could be of fungal origin. During an examination of a sample of toxic Brazilian peanut meal for the presence of fragments of poisonous plants, it was noted that the pieces of peanut cotyledon tissue contained hyphae although none was present in a sample of nontoxic meal (Austwick and Aycrst, 1963). Although attempts at culture showed that these hyphae were dead, Sargeant *et al* (1961) subsequently succeeded in producing pure cultures of certain of the fungal species present in a highly toxic sample of peanuts from Uganda heavily contaminated with fungi. A chloroform extract of a culture of one of the isolates grown on Czapek's solution agar was found to contain a fluorescent material when chromatographed on paper which was toxic to ducklings and produced the characteristic symptoms associated with Turkey 'X' disease. The toxin producing fungus was identified as *Aspergillus flavus* Link ex Fries (Sargeant *et al,* 1961) and the toxin was given the name 'aflatoxin' in view of its origin. Following this discovery, many strains of *A. flavus* were examined for toxin production and it was soon clear that Turkey 'X' disease was initiated by the aflatoxin.

1.3 Types and Structures of Aflatoxins

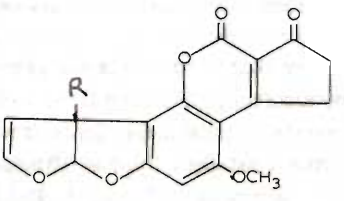
1.3.1 Structure and Chemistry of the Aflatoxins

The aflatoxins are a group of acutely toxic and highly carcinogenic mould metabolites produced by *Aspergillus flavus.* The toxins have closely similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds (see Fig. 1.1 below).

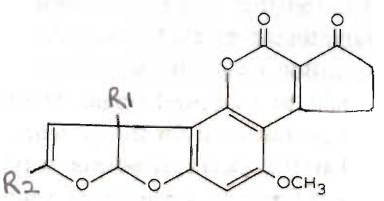
These are colourless to pale yellow crystals, intensely fluorescent in UV light, emitting blue or yellow-green fluorescence, from which the designations "B" and "G" were derived (IARC, 19721). Detail physico-chemical properties of these compounds, such as obtained from m.p., UV, IR, NMR and MS studies are given in Buchi and Rae (1969). Tables of these properties are also given in IARC (1972) and Heathcote and Hibbert (1978a).

1.3.2 Commonly Encountered aflatoxins : B1, B2, G1, M1, M2

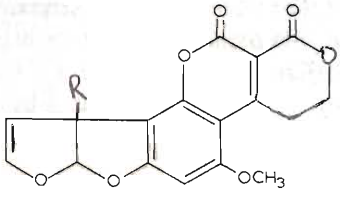
Nesbitt *et al* (1962) and Hartley, Nesbitt and O'Kelly (1963) showed that the single blue-fluorescing spot of toxin-containing extracts observed by Sargeant *et al* (1961), could be split into four main components when the extracts were chromatographed on thin-layer silica plates developed in chloroform-methanol. Two of these components with Rf values of 0.4 and 0.36, fluoresced blue under UV light and were designated aflatoxins B1 and B2 respectively; the



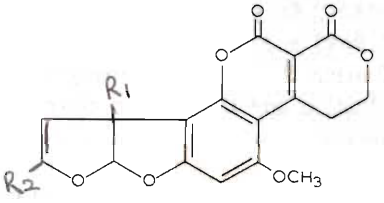
R = H Aflatoxin B1
R = OH Aflatoxin M1



R1 = H, R2 = H Aflatoxin B2
R1 = H, R2 = OH Aflatoxin B2a
R1 = OH, R2 = H Aflatoxin M2
R1 = OH, R2 = OH Aflatoxin M2a



R = H Aflatoxin G1
R = OH Aflatoxin GM1



R1 = H, R2 = H Aflatoxin G2
R1 = H, R2 = OH Aflatoxin G2a
R1 = OH, R2 = H Aflatoxin GM2
R1 = OH, R2 = OH Aflatoxin GM2a

Figure 1.1 Structures of Aflatoxins

two with slightly lower Rf values of 0.34 and 0.31 fluoresced turquoise under UV light and were designated aflatoxins G1 and G2 respectively. The molecular formulae for aflatoxin B1 and aflatoxin G1 were deduced in 1962 by Nesbitt and co-workers from elemental analyses and mass spectral data to be $C_{17}H_{12}O_6$ and $C_{17}H_{12}O_7$ respectively.

That aflatoxin B2 is the dihydro-derivative of aflatoxin B1 was shown in 1963 by Van der Merwe, Fourie and de Scott who synthesized aflatoxin B2 by the catalytic hydrogenation of aflatoxin B1 with the uptake of one molar equivalent of hydrogen. These investigators also showed that aflatoxin G2 was the dihydro-derivative of aflatoxin G1 in the same manner and put forward tentative structures for aflatoxins B1 and G1.

The isolation of these four main aflatoxins, the structure of which are

shown in Fig. 1.1, was soon followed by the isolation of numerous other analogues. This was facilitated by the fluorescence of the compounds in UV light and by their ability to be extracted into a variety of organic solvents; there were also fairly rapid improvements in the separation techniques.

When aflatoxin B1, or an unseparated mixture of the aflatoxins, is fed to animals certain related toxins called 'milk toxins' may be recovered from the secretions of the subject. Allcroft *et al* (1966) later showed by TLC and UV studies that a similar factor, a blue-violet fluorescing compound which had an Rf value well below that of aflatoxin B1, appeared in the urine of sheep when fed mixed aflatoxins, which they believed to be probably identical with the milk toxin, and these workers suggested the trivial name 'aflatoxin M' for the substance.

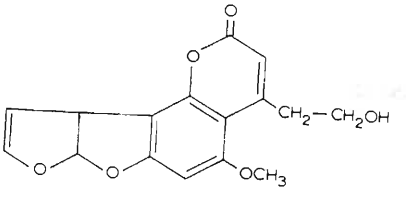
In 1967 Masri *et al* reported the identity of aflatoxins M1 and M2 from several sources and also detected the toxins among the metabolic products of *Aspergillus flavus*. They also reported on their structures. The molecular formula, $C_{17}H_{12}O_7$, of aflatoxin M1 contained one more oxygen atom than that of aflatoxin B1. On the basis of spectroscopic evidence, it was postulated that the M1 aflatoxin was the hydroxylated derivative of aflatoxin B1 with the hydroxyl group in the C-4 position of the terminal furan ring, and that aflatoxin M2 was the dihydro-derivative of aflatoxin M1 as shown by hydrogenation of aflatoxin M1 in acetic acid using a palladium catalyst. Structures of M1 and M2 aflatoxins are as shown in figure 1.1.

Many investigators have examined the biological effects of these aflatoxins. Results have shown that they are lethal to various animals and that aflatoxin B1 was the most acutely toxic, followed by M1, G1, M2, B2 and G2, in the order of decreasing potency. The oral LD50 (expressed in μg per 50g body weight, as determined in day-old ducklings) (as compiled by Jones, 1975) are B1:18.2, M1: 16.6, G1: 39.2, M2: 62.0, B2: 84.8 and G2: 172.5. It has also been shown that some animals were appreciably more susceptible than others; ducklings appeared to be the most vulnerable. A cause-and-effect relationship between aflatoxin consumption and development of liver cancer had been unequivocally demonstrated in at least 8 animal species including non-human primates (Campbell and Stoloff, 1974; FAO, 1977; Tulpule and Bhat, 1978). Reviewing the biological (genetic and non-genetic) responses of this mytocoxin on a wide variety of systems, Legator (1969) reported that evidences appeared to support the conclusion that the genetic effects of aflatoxin can be expressed in terms of carcinogenic, teratogenic and possibly mutagenic. It was emphasized that the potential hazard of aflatoxin to man is not its acute toxicity, but these potential genetic effects.

1.3.3 Other aflatoxins

The late 1960's saw the discovery, identification and characterisation of

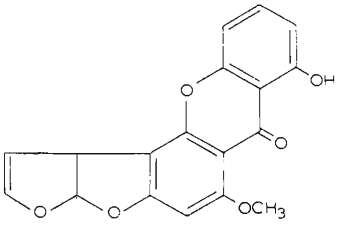
several other analogs of aflatoxins, GM1 and GM2 are hydroxylated derivatives of aflatoxins G1 and G2 respectively, whilst M2a and GM2a are dihydroxy aflatoxins. Two other hydroxylated aflatoxins are B2a and G2a. Yet another blue-fluorescing compound isolated was aflatoxin B3. The structures of these various aflatoxins are given in figure 1.1 and 1.2. Detail discussions on the discoveries and identification of these toxins are given by Heathcote and Hibbert (1978b). The discovery of yet other aflatoxins such as aflatoxicol, aflatoxin P1 and aflatoxin Q_1 are described in Heathcote and Hibbert (1978c) and Hirono (1981).



Aflatoxin B3 Figure. 1.2

1.3.4 Some related metabolites

One of the earliest metabolites to be isolated, which possessed a formula closely analogous to the aflatoxins and contained the bis-dihydrofuran-ring system, was sterigmatocystin, (Fig. 1.3) isolated from cultures of *Aspergillus versicolor.*



Sterigmatocystin
Fig. 1.3

A series of important mycotoxins named collectively as ochratoxins (Fig. 1.4) are produced by the species of *Aspergillus* known as *A. ochraceus.* Ochratoxin A was found to be acutely toxic to ducklings and caused severe liver lesions in weaning rats (Pitout, 1968).

Ochratoxin A: R = H, R′ = Cl
Ochratoxin B: R = H, R′ = H
Ochratoxin C: R = C_2H_5, R′ = Cl

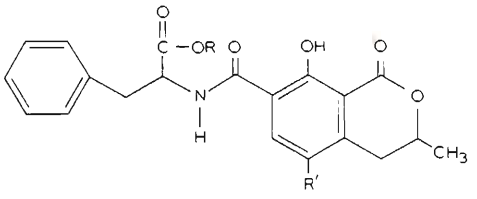


Fig 1.4

A detailed discussion of these and other mycotoxins are given in e.g. Wilson (1966), Purchase (1974) and more recently in two volumes on mycotoxins edited by R.C. Shank (1981).

1.4 The Assay of Aflatoxins

For the assay of the aflatoxins, two principal methods have been developed, i.e. physico-chemical assay and biological assay. For an exact measurement of the type or quantity of aflatoxin present in a given commodity, physico-chemical assay, which is based on the fluorescence of the aflatoxins under ultraviolet (UV) light, is used. Biological assays are the only means of determining the level of effective toxicity in cases of suspected contamination, but they are time consuming and do not provide a reliable means of distinguishing the type of aflatoxin present.

1.4.1 Physico-Chemical Assay

The actual assays per se may be rather straight forward, but since the aflatoxins are present only in trace amounts, even in heavily contaminated agricultural commodities, it is often necessary to extract, purify and concentrate them beforehand. For instance in food items such as groundnuts, soyabeans, cereal grains, coffee or cocoa beans, which contain large amounts of natural lipid components, these must first be removed to prevent their interference with the assay. Because the aflatoxins were first associated with mouldy peanuts, most of the early procedures for their extraction were developed with this commodity in mind. Soon after realising the diverse nature of contaminated foods, numerous methods of extraction and purification have been proposed, and these are frequently changing and improving. Most of these methods, however, are modifications of early methods. The manual by the Tropical Products Institute (TPI) (Jones, 1972) and Heathcote and Hibbert (1978d) have described in some detail the necessary treatment procedures for various foodstuffs. Basically, the methods involve an initial defatting procedure (if necessary), followed by extraction with organic solvents, and a "clean-up" or purification process, often by column chromatography, and finally concentrated to a suitable volume for subsequent analysis.

The "cleaned-up" food extract thus otained is then chromatographed to separate the aflatoxins. Paper chromatography was used by Sargeant *et al* (1961) in the first aflatoxin assay procedure reported. This was soon superseded by the more discriminating TLC methods. Initially alumina was used, but it was soon realised that silica gel greatly improved the TLC resolutions. Details of the various fine points of the development of the TLC procedures have been described by Pons and Goldblatt (1969) and Heathcote and Hibbert (1978d). So as to enable better quantitation of the aflatoxins, some laboratories have adopted High Performance Liquid Chromatographic (HPLC) procedures for the separation of these toxins. A number of these procedures have recently been developed for the analysis of various foodstuffs (e.g. Stubblefield and Shotwell, 1977; Beebe, 1978; Pons and Franz, 1978 and Pons, 1979).

The aflatoxins thus separated are then identified and quantitated by various physico-chemical methods. The methods available range from simple detection to highly sophisticated spectroscopic techniques. As mentioned earlier, the most useful and characteristic property of the aflatoxins is the intense blue or blue-green fluorescence which they emit when exposed to UV light. Hence fluorescence under long-wave UV light forms the basis of the many techniques which have been devised for their assay, whether on paper, thin-layer or column. Heathcote and Hibbert (1978d) have described these in detail. The limits of detection could range from 0.4 ng using visual and densitometer detection, to 1.0 ng using UV and fluorescence detecting instruments and 0.03 ng if mass spectrometry is used (Coker, 1979). An important recent development in methodologies is the use of rapid screening methods using "mini-columns". These are particularly useful in the food industry, where dealing with agricultural products on a large scale, inspectors need to be able to examine a commodity and make a decision on the fate of a batch, based on quantitative assessment of contamination.

1.4.2 Biological Assay

The susceptibility of ducklings to aflatoxins was made the basis of the first biological assay devised for the aflatoxins by Asplin and Carnaghan (1961). The use of day-old ducklings, combined with chemical identification of the mycotoxin has become the most widely used and accepted procedure for aflatoxin identification. This has been attributed to the sensitivity of the duckling to aflatoxin injury and the almost immediate induction of a somewhat specific response, bile duct proliferation (Legator, 1969).

The injection of aflatoxin into embryogenated hen's egg was proposed by Verett, Marliac and McLaughlin (1964) as the basis for a more sensitive test than the duckling test. Being simple, reproducible and sensitive, the chicken embryo technique has been said to offer several advantages for bioassay of aflatoxins (Legator, 1969). The use of other animals, such as the rainbow trout,

rat and mice, hamster and guinea pig, monkey and even plants were also reviewed by this author. The use of cell-free systems, microorganisms and cell cultures were also described in detail.

1.4.3 Assay of Aflatoxins in Malaysia

The earliest reports on the assay of aflatoxins in foods in the country were those of Chong and his co-workers at the Institute for Medical Research (Chong and Beng, 1965; Chong, 1966 and Chong *et al,* 1966). The method used was essentially that developed by the Tropical Products Institute, London (1964). Foods were ground and then defatted in a soxhlet extractor with methanol or petroleum ether. Aflatoxin in the defatted material was then extracted into chloroform, which was then reduced to a suitable volume for chromatography. Separation of aflatoxins was done by TLC using kieselgel. Visual estimation of the aflatoxin content was made by comparing with reference standards developed on the same TLC plate.

Following the procedure described above, Chong *et al* (1966) were unable to detect the presence of aflatoxins in the moulded soya beans used in the manufacture of soya sauce, although they were able to isolate the fungus *A. flavus* from the beans. Using the day-old duckling bioassay method to re-examine these products, they were able to rule out firmly the possibility of aflatoxin contamination in the moulded soya beans (Chong and Ponnampalam, 1967).

Lim and Yeap (1966) also reported on the analysis of aflatoxin around the same time, but on feedstuffs. After defatting, extraction and concentration, the extracts were separated using paper chromatography. Semi-quantitation was done by visual comparison with reference standards.

In the surveys of mycotoxins in foods reported by Saito and Bhagwan Singh (1975), several toxins were examined by TLC (using silica gel) after the necessary preliminary treatments. A toxicity bioassay of the food extracts was also carried out using HeLaS3 cells. Whereas no mycotoxins were detected in the rice samples using chemical analysis, cytotoxic effects were observed for the cultured cells. Some toxic effects were observed for the cultured cells. Some aflatoxin producing strains of *A. flavus* and sterigmatocystin producing strains of *A. versicolor* were also found in the isolates collected from these rice samples.

More recently, in the studies of some 300 food samples, the IMR (1978) had used a method based on a revised and up-dated procedure of TPI (Jones, 1972) for the detection and semi-quantitation of aflatoxins. In the study of rice samples from various National Rice and Padi Board field stations, MARDI (1983) reported the experiences of using "mini-columns" for the analysis of these mycotoxins.

1.5 Occurrence of Aflatoxins

Although the first known natural occurrence of aflatoxins was associated with peanuts, it is now known that the *A. flavus* group of moulds is ubiquitous and capable of development over a wide range of temperature on substrates of high carbohydrate content. Hence many agricultural commodities and their products are vulnerable to contamination. The presence of aflatoxins in biologically significant levels in food samples collected from various parts of the world has been discussed by Wogan (1968). The food samples covered a wide variety of agricultural commodities including barley, beans, corn, cotton seed, rice and wheat. Groundnut, groundnut products and corn appear to be the most susceptible to be contaminated. Incidences of contamination as well as aflatoxin levels vary greatly. Levels close to 1 ppm are common for groundnut and groundnut products; levels over 5 ppm have also been reported. Aflatoxin B1 is most frequently present in contaminated samples; B2 and G1 are present much less frequently. It is in fact now clear that mycotoxin contamination is a global problem. From surveys conducted in several countries as part of the FAO/WHO/UNEP project, mycotoxins were said to have been detected in 7 major cereal grains, 5 important oilseeds, 6 tree nuts, vegetable oils, pulses, root crops, other vegetable products and animal products including milk, cheese, meat and fish (FAO, 1977).

Since the chief habitat of these fungi is the soil, any agricultural crop, particularly sub-soil crops, cannot escape fungal contamination. At various stages of growth, harvest and storage, these crops provide good substrates for fungal growth, although the main problem seems to be at the stages of drying and storage. The various factors influencing fungal growth and mycotoxin development have been extensively described by various authors including Diener and Davis (1969), Heathcote and Hibbert (1978e) and FAO (1977).

Other possible sources of food contamination are the meat and milk of animals which have been fed rations contaminated with aflatoxin. Mould contamination of certain moist foods, especially during storage, have also been reported, for instance by airborne fungal spores. In addition, certain fungi are used in the manufacture of specific food items for human consumption, such as the many fermented foods eaten in this region. It is therefore necessary to carefully examine these foods for the presence of any toxigenic fungi.

It is well known that the presence of the mould does not necessarily indicate that aflatoxins will be present, since many of the strains of *A flavus* do not produce toxins. It has been estimated that of the 1400 strains of the mould isolated from different sources, only 58% produced aflatoxin (FAO, 1977). However, it must be emphasized that the absence of visible indicators of mould growth on a foodstuff does not necessarily mean that the toxin too will be absent.

1.5.1 Occurrence of Aflatoxins in Malaysian Foods

There has not been many reports on the occurrence of aflatoxins in local foods. However, some important studies have been carried out. These will be briefly reviewed in this section.

It appears that the first suggestion on the occurrence of the aflatoxin problem in Malaysia was by Lim (1964) when he reported an outbreak of a disease which had occurred in 1960 on two pig farms in Malacca. The disease which manifested with gross liver damage, was said to be associated with the introduction of diets containing peanut meal or cake from Thailand. Later, Lim and Yeap (1966) reported the detection of aflatoxins in various feed ingredients imported into the country, including several types of oil cakes and meals.

The first chemical identification and analysis of aflatoxins in local foods for human consumption was however published by Chong and Beng (1965), in which samples of groundnut oil for cooking were examined. It was found that 5 out of the 7 samples of unrefined oil were contaminated with aflatoxins B1 and G1, with levels ranging from 8 ppb to 16 ppb. Their study also confirmed that refinement of the crude oil removed the toxins and rendered the oils harmless since they were not able to detect aflatoxins in the samples of refined groundnut oil examined.

Chong, of the IMR, and his co-workers continued to report on the occurrence of this mycotoxin in local foods. A study of samples of groundnuts (shelled as well as unshelled), groundnut cooking oil and peanut butter from various sources was reported by Chong in 1966. Ten of the 38 samples of the shelled nuts contained aflatoxin B1 at levels ranging from 0.05 – 2.0 ppm. It was also noted that not all of the contaminated nuts were visibly mouldy. No aflatoxins were detected in the 10 samples of local "Menglembu" type of unshelled groundnuts examined. Of the 28 samples of unrefined oils studied, 23 were found to be contaminated with aflatoxin B1 up to a maximum level of 0.008 ppm. In some of the contaminated nuts and oils mentioned above, aflatoxin G1 was also detected, often in about equal amounts as the B1. All except one of the refined cooking oils examined were free of aflatoxin contamination. Three of the 16 samples of peanut butter investigated were also found to be contaminated with aflatoxin B1.

Examination of moulded soya beans used in the manufacture of soya sauce, and soya sauce itself by Chong *et al* (1966) using chemical methods revealed no aflatoxin contamination of these products. Chong and Ponnampalam (1967) were able to confirm this finding using the day-old duckling bioassay procedure.

Some years later, a team of Japanese investigators, in collaboration with the IMR conducted a fairly comprehensive survey of mycotoxin contamination of some 275 food items consumed by the communities in Sekinchan, Kuala Selangor (Saito and Bhagwan Singh, 1975). Grains, beans and legumes were the

most exensively sampled as these were found to be the major components of the diets of these conmunities through an initial dietary survey. Other foods examined included curry powder, dried fish and shrimp. Reporting on the mycoflora of these foods, it was noted that rice samples harbored the greatest number of different fungi, with *Aspergilii* the chief species encountered. Rice samples were collected from the area and together with other samples bought in markets, a chemical analysis and bioassay based on cultured cells were carried out. The toxins investigated were aflatoxins, sterigmatocystin, ochratoxin A, luteoskyrin, rugulosin, patulin and penicillic acid. However, only aflatoxins was detected for the samples investigated. No mycotoxins were detected in the rice samples, the moulded soya beans and the tempeh sample, although bioassay indicated some cytotoxic effects in the rice samples. Aflatoxin was detected in some of the groundnut samples studied.

The IMR continued to play a major role in the detection of aflatoxin contamination of local foods. A study of 329 foods, comprising a wide variety of commonly eaten items such as groundnuts and groundnut products (including raw, salted, "Menglembu" nuts, peanut butter, satay sauce, groundnut candy and biscuits), rice, soya sauce, fermented soya products, soya flour, wheat flour, corn flour, chilli sauce, dried chillies, dhall, preserved fruits, dried fish, *belacan* and shrimp paste has been reported (IMR, 1978). Frequency of contamination was reported to be very low, with levels ranging from 0.05 ppm to 1.0 ppm, and was restricted to the presence of aflatoxin B1, principally in groundnut and groundnut products.

Further analysis of rice samples has been reported by MARDI (1982) on aflatoxin contamination of samples from 4 different National Rice and Padi Board stations at 2 monthly intervals throughout 1982. No aflatoxin contamination was detected. However, *A. Flavus,* including the toxin producing strains were isolated. It was suggested that any further increase in moisture content of the stored rice could have resulted in aflatoxin contamination.

1.6 Epidemiological Studies

A vast amount of literature concerning the metabolism, pharmacology, animal toxicity, and carcinogenic activity of aflatoxins had already accumulated less than 10 years after its discovery in 1961. The effects of these toxins in man and the amounts chronically ingested with the diet was, on the other hand, not well established. However, soon after the dramatic outbreak of turkey 'X' disease in Britain and the realization that a fungal metabolite was involved, the possibility that contamination of dietary staples by aflatoxins could be an aetiological factor in liver cancer was already suggested. Oettle (1965), reviewing the then available experimental and early epidemiological evidences, concluded that a mycotoxin hypothesis fitted the known liver cancer data better than any other

suspected aetiological factors. Incidents of isolated cases of human aflatoxicosis reported by various investigators have been discussed by Campbell and Stoloff (1974).

Several recent epidemiological studies, where estimates of aflatoxin intake by populations and the incidence or prevalence of primary liver cancer were simultaneously determined, have been undertaken to further test this "aflatoxin hypothesis". The often quoted studies are those conducted in Africa and Thailand. Some of those reported in the 70's are cited here.

Alpert and co-workers had noted that areas of high hepatoma incidence included all the African countries south of the Sahara (especially amongst the black Africans), Southeast Asia and probably also southern India, China, Korea and Japan. Further, it was noted that Africa and Asiatic people who have moved to industrialized countries of low incidence tended to acquire the low hepatoma rate of their adopted country. It was thus suggested that environmental factors were important causative agents rather than simple genetic or racial predisposition (Alpert and Davidson, 1969). These investigators had observed that in Uganda, a country with a well-documented high incidence of liver carcinoma (Alpert, Hutt and Davidson, 1968), aflatoxin was detected in 40% of foods randomly sampled from houses (Alpert, Davidson and Wogan, 1968). In order to determine if there was indeed a correlation between these factors, a survey was carried out to determine the frequency and amount of aflatoxin contamination of foods in Uganda and to compare these data with the incidence of hepatoma over a 3–year period in several racial and tribal groups in the country (Alpert *et al,* 1971). It was found that aflatoxin contamination was greatest in areas with high incidence of liver cancer. It was felt that the presence of aflatoxins in foods could account for part of the increased incidence of hepatoma in Uganda and perhaps elsewhere.

At the same time, Keen and co-workers had examined the recently established cancer registry and noted that primary liver cancer was a serious problem in Swaziland, also in Africa. A similar study was carried out and it was found that the geographical distribution of contaminated samples corresponded, to a large extent, with that of the geographical distribution of primary liver cancer (Keen and Martin, 1971a).

Further evidence that aflatoxin may be involved in the aetiology of primary liver cancer was reported by Peers and Linsell (1973) in a study in another African nation, Kenya. To further test the strength and consistency of the association observed in Kenya, a second study was carried out by Peers and co-workers in Swaziland. It was felt that the data of Keen and Martin (1971a, b) and other previous investigators on stored groundnuts or market samples did not adequately indicate dietary exposure levels of the toxin. Hence analysis was performed on prepared meals ready for consumption by the households (this was also done for the 1973 study). It was reported that the results obtained were consistent with the hypothesis that chronic aflatoxin ingestion was re-

lated to the incidence of liver cancer within an African population (Peers, Gillman and Linsell, 1976).

Evidences from yet another African country, Mozambique, was provided by the study of Van Rensburg *et al* (974). Aflatoxin intake was also estimated from prepared foods "from the plate". The results reported were said to be the highest, both for primary liver cancer rate and known aflatoxin intake. In an attempt to illustrate a dose-response relationship, data from other investigators and those obtained from this study were pooled and standardised. It was shown that cancer incidence appeared to be linearly related to the logarithm of the level of aflatoxin intake; toxin intake range from 3.5 to 222.4 ng/kg body weight/day and cancer rates were from 0.7 to 25.4 x 100,000 per year.

Detailed investigations were undertaken to study the outbreak of acute hepatitis in tribal areas extending over 200 villages in Western India in 1974. The staple food of the people in the area, maize, was found to be heavily contaminated with aflatoxin, at levels ranging from 6.5 – 15.5 ppm. Based on epidemiological, mycological, mycotoxic and histopathological considerations, it was concluded that the disease was caused by consumption of the contaminated maize (Krishnamachari *et al,* 1975).

Similar evidences were obtained from studies in Thailand and described in a review by Wogan (1975) of the epidemiological studies described above. More recent reviews are those of Linsell and Peers (1977a, b). It must however be emphasized that these field studies showing an association between cancer rate and toxin intake do not constitute proof of a causal relationship.

It has in fact been pointed out that there are uncertainties and complicating factors in the observed association which have to be taken into account. Cambell and Stoloff (1974) had analysed and discussed some of these in their review of some of the reported epidemiological studies. Earlier, Wogan (1968) had cited the example of the apparently low incidence of liver carcinoma in areas of Central and South America with climatic and other conditions seemingly as conducive to mould spoilage of foodstuffs as those in Africa and Southeast Asia, where incidence is high. More recently, it has been noted (Foster, 1982) that in the Southeastern part of the United States, where peanuts and corn (both known to be highly susceptible to aflatoxin contamination) are important items in the diet, the incidence of human liver cancer is not higher than that of the rest of the country. It is now clear that liver cancer like other cancers, is a multistaged and multifactorial process. Hence other factors such as age, sex, nutritional status, concurrent exposure to other agents, genetic factors and concurrent illnesses may play a part in the process.

Recent evidences seem to show that the cause of human liver cancer involves more than aflatoxin ingestion; prior exposure to or simultaneous exposure with hepatitis B virus (HBV) appears to be a prerequisite. It has been pointed out that hepatitis B is more common in developing countries and that the carrier state is associated with liver cancer (Linsell and Peers, 1977b). A strong associa-

tion between liver cancer and HBV infection is said to have been noted in several studies undertaken in many countries (Campbell, 1982). In addition, it has been suggested that factors affecting the course of events after the neoplastic lesion has been initiated by aflatoxin may be even more important than the amount of cancer cells initially formed. Investigations in the laboratory of T.C. Campbell have suggested that dietary protein intake plays a significant role in this post-initiation phase. Campbell (1982) had even suggested the possibility that nutrient intervention in the later stages of post-initial period can either turn on or turn off the tumour progression process.

Evidences accumulated so far thus seem to justify continued investigations into effective measures for the monitoring and control of aflatoxin contamination. Absolute proof that aflatoxin is a hepatocarcinogen in man is not likely to be forth coming and it is generally felt that all attempts to reduce fungal infestation and toxin contamination can only be beneficial.

1.7 Control of Aflatoxin

The problem of mycotoxin contamination has serious and far-reaching effects. With regards to health and toxicological aspects, a broad spectrum of diseases, both acute and chronic, affecting humans and livestock in both industrialized and developing countries are known to have resulted from the consumption of contaminated foods. The toxins could reduce the availability and certainly the quality of food supplies in many parts of the world, the effects of which would be especially felt in developing countries facing problems of over population, hunger and malnutrition. In addition, mycotoxin contamination has serious trade and economic repercussions. Thus national governments as well as international agencies such as FAO and WHO have been taking definite steps to prevent and control mycotoxin contamination. Scientists of various disciplines from 40 countries gathered in Nairobi for a joint FAO/WHO/UNEP conference to discuss the mycotoxin problem, including and indeed emphasising the control measures to be taken (FAO, 1977).

As contamination could take place at any one of the various points in the food chain from production through harvesting, storage and processing to consumption, measures to be taken against mycotoxins would necessarily have to be concerted efforts of various disciplines. These would include better in-field protection of crops, improved agricultural and animal husbandry practices, use of appropriate post-harvest technologies, better processing, storage, marketing and distribution systems. To enable a continual assessment and monitor of the problem and to facilitate further priorities and actions, programmes of surveillance should be established to determine the incidence and site of mycotoxin contamination in the food chain. The report of the FAO conference on mycotoxins mentioned above (FAO, 1977) has discussed and given guidelines

for some of the measures to be taken for the prevention, monitor and control of mycotoxin contamination.

Although prevention is the preferred approach to the problem of mycotoxin contamination of human foods and animal feeds, decontamination or detoxification procedures could salvage contaminated products and render them available for consumption. A wide range of methodologies have been developed, such as by extracting the toxins from the foods using various solvent systems, or by destruction of the toxins "in situ" by e.g. heat, ionising radiation, biological degradation or chemical inactivation using oxidising agents, acids or alkalis. These have been described at length by Heathcote and Hibbert (1978f) and Jones (1975). The method (s) of choice would necessarily depend on the nature of the commodity and the scale of operation. Clearly, the process should be economical so that the decontaminated product would not be still unavailable to the people, now because of its price. In addition, the process has to satisfy certain technical criteria. Ideally it should destroy, inactivate or remove the mycotoxins, fungal spores as well as mycelia, whilst retaining the nutritive value and acceptability of the commodity.

Available data on possible human and animal health hazards associated with aflatoxin contamination of foods and feeds have prompted several countries to establish and enforce aflatoxin regulatory programmes for some domestic and imported commodities. These are mainly for groundnuts and groundnut products, and in crops harvested or stored under conditions likely to result in fungal infection. Some form of regulation or guideline for foods and/or feeds are said to be in force in Brazil, Canada, Denmark, England, European Economic Community countries, Germany, Hungary, India, Italy, Japan, The Netherlands, South Africa, and the United States. (Campbell and Stoloff, 1974; Jones, 1975; Coker, 1979). It is clear that it is no easy task to establish appropriate aflatoxin limits for foods and feeds. These limits or tolerances would depend on health effects of the toxins, the effects of enforcement on food and feed availability, and on the limits of sensitivity of available analytical methodologies. Obviously, it would not be practical to have a specific single aflatoxin limit or tolerance. The definition of 3 distinct and general categories of food and feed usage, each with its own acceptable levels of contamination has been recommended (FAO, 1977): I. commodities for direct human consumption; II. commodities for use as ration of dairy cattle and other animals kept for milk, and for starter rations; III. commodities intended as feed for all other livestock and poultry. The limits would be the lowest for commodities in catetory I, intermediate in II and highest in III. It is to be expected that as more comprehensive information becomes available, there will be increased regulatory activities around the world. Regulatory programmes may even be extended to cover other mycotoxins, for which no controls are presently known to exist.

In Malaysia, soon after the discovery and world-wide as well as local reports of the aflatoxin problem, the Malaysian Scientific Association set up a com-

mittee in 1966 to encourage investigations into aflatoxin contamination of locally available foods and to formulate recommendations for the reduction of hazards arising from aflatoxin in the country (Moir, 1968; Chong, 1977). The committee had recommended to the Ministry of Health that Malaysia should adopt legislations to make the import or sale of foods contaminated with aflatoxins illegal. However, no definite official actions were said to be taken by the authorities.

After some years, being better equipped to tackle the problem, the Ministry of Health initiated the need for regulatory control of aflatoxins in the newly drafted Food Regulations through the control of allowable limits of the toxin in foods. To establish baseline data, a survey was conducted by the Ministry in March 1982 to determine the levels of contamination of over 200 food items. Realising that measures to curb the problem requires an integrated multisectoral approach, coordination and collaboration was sought from various agencies including the Departments of Agriculture, Chemistry and Veterinary, MARDI, SIRIM, IMR, and the Ministry of Trade and Industry to arrive at an effective surveillance and control programme (Ministry of Health Malaysia, 1983).

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2 LEAD

2.1 Introduction

Lead, Pb, atomic weight 207.19, atomic number 82, is in the periodic system group IVB. It is a soft, bluish-grey, malleable metal, ubiquitous in the environment, occurring in nature chiefly as the sulphide ore known as galena (Ratcliffe, 1981). Lead is the most abundant of the heavy metals in the earth's crust. It is also one of the oldest metals known to mankind, as evidenced by discoveries of artifacts made from lead that date back to some 3000 years B.C, (IARC, 1980).

Due to its useful physical properties, such as those of comparative ease of extraction from the ore, mallebility, ductility, corrosion resistance and poor conductance, elemental lead has had a multiplicity of uses for over 3000 years (Ratcliffe, 1981). The ancient civilizations of Phoenicia, Egypt, Greece, India and China have been known to have smelted and used lead for vessels, roofs, water ducts, utensils, ornaments and weights, whilst the Romans used lead extensively in the transport of water and the storage of wine and food. The coloured oxides were also said to have been used as pigments in cosmetics and glazes. Grandjean (1975) has given an interesting account of these earliest uses of lead. The use of lead has increased tremendously since the commencement of the industrial revolution. Substantial benefits to modern-day society have been found for the use of this metal in a host of industrial and commercial applications. Estimates in the mid-70s have indicated that the largest consumer of lead was the lead-acid storage battery industry, followed by tetraalkyllead production as an "anti-knock" additive in petroleum (Tsuchiya, 1979; Harrison and Laxen, 1981; Ratcliffe, 1981).

Unfortunately, the mining, smelting and refining, as well as the production and use of lead-based products have given rise to the release of lead into the environment (Harrison and Laxen, 1981). The amount of lead so mobilised into the biosphere has reached a point where lead represents one of the most ubiquitous of all metal contaminants. In view of such widespread distribution, it is not surprising that lead is one of the most abundant of the non-essential trace elements in the body (Biddle, 1982).

As a result of this, there has been considerable contamination to the environment, to the food we eat, the water we drink and the air we breathe. Over the centuries, many people have been poisoned by this technologically useful metal (Mahaffey, 1978). Lead has been known for centuries to be a cumulative metabolic poison (Posner, Damstra and Nriagu, 1978). A voluminous literature now exists on the toxicity of the metal. More has probably been written on the

toxicity of lead than any other heavy metal (Boline, 1981). It might be said that no other pollutant has caused so much alarm in recent times as lead. With the finding that in high doses it can cause "brain damage" in children, the problem has provoked an emotional, aside from an intellectual reponse to its presence in the environment (Lawther, 1978).

With increasing industrialisation and urbanisation in Malaysia, it can be anticipated that lead intoxication will become an increasing problem in the country. It is thus felt that a review of current knowledge could contribute to our understanding on the topic. This paper reviews where this pollutant could possibly arise from, its metabolism in the body, the indices which may be used to diagnose lead poisoning, the toxicological effects that may be brought about, and the control strategies to be considered in tackling the problem. In those areas where Malaysian studies are available, these will be cited.

2.2 Sources of Lead Exposure

Lead has been described as a multimedia pollutant, meaning that there are multiple sources of lead exposure within the environment (WHO, 1977). Most of these sources are summarised in Figure 2.1. There is a continuing debate as to the relative significance of each of these pathways. This is unlikely to be readily resolved due to the diversity of the sources of exposure and their lead concentrations, as well as the varied nature of the individual's metabolic response to the lead (Harrison and Laxen, 1981). Although there is a long history of human exposure to lead, the relative importance of the different pathways of lead exposure must have altered in recent times. Most investigators felt that for modern man, the greatest exposure to lead among adults not employed in lead-related industries and among children without pica (the compulsive eating of non-food substances such as soil, paint chips or plaster) is through foods and beverages (Harr, 1975; Mahaffey, 1978; Tsuchiya, 1979; Moore, Meredith and Goldberg, 1980; Biddle, 1982). In specific circumstances, usually involving industrial exposure, the contribution of airborne lead may become important. This section will hence deal in some detail the contribution of lead in food to man's exposure to the metal, followed by a brief discussion of lead in water and lead in air.

2.2.1 Lead in Food

2.2.1.1 Sources of Lead in Food

It is first important to understand the various sources of lead in the foods that we consume. Lead residues may occur in food as a result of (a) biological uptake of the metal naturally present in the soil or of lead added to the soil;

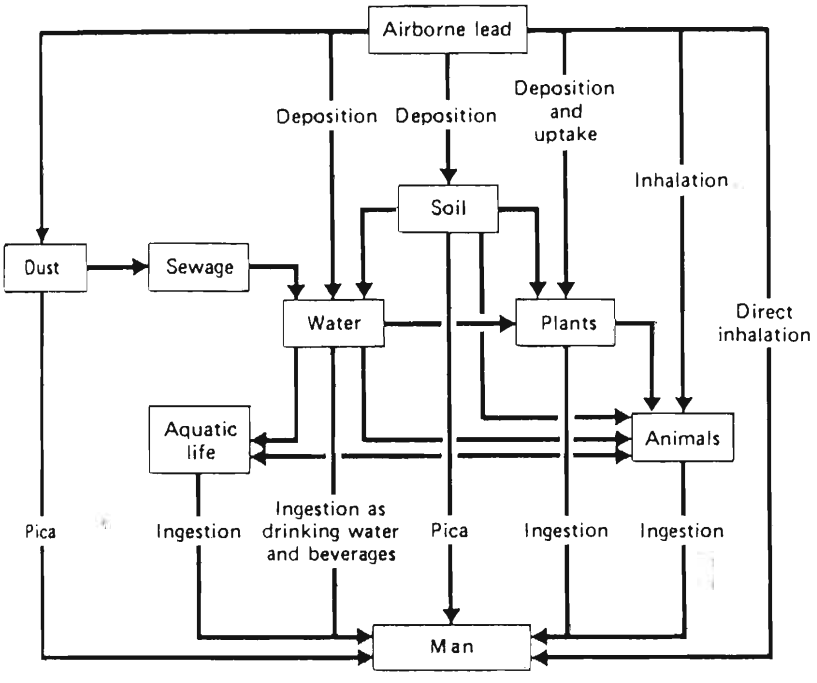

Figure 2.1 Sources of Exposure to Lead (Source. WHO, 1977)

(b) deposition of airborne lead onto crops or herbage and ingested directly (as consumer crops) or indirectly (e.g. as meat) through the food chain; and (c) inadvertent addition of lead during food processing, food preparation and cooking using lead-containing vessels, cans, utensils, or water (Harr, 1975; Mahaffey, 1978; Ratcliffe, 1981).

Lead is naturally present in the soil, with a range in agricultural soils of about 2 to 200 μg/g (Harr, 1975). Many variables are undoubtedly important in controlling the release of lead from the soil to the crops. including concentration of lead in the soil, soil composition, pH and other as yet undefined variables (Harr, 1975). Treatment of land with industrially contaminated sewage sludge or lead-containing chemical fertilizers and pesticides may lead to higher lead content of vegetables, herbage and meat from livestock grazed on such treated land (Ratcliffe, 1981).

There appears to be considerable controversy regarding the contribution of fall-out lead (foliar deposition of atmospheric lead), particularly from motor vehicles, to the lead content of the foods. Ratcliffe (1981) however felt that it is reasonable to conclude that in the case of grasses, vegetative parts of cereals and leafy crops, the bulk of the lead content is attributable to fallout from atmos-

pheric lead. Some of this may be removed during preparation and washing (Low and Lee, 1979; Harrison and Laxen, 1981). On the other hand, much less of the lead content of grains, fruits and roots crops is likely to be attributable to direct aerial contamination, compared with soil lead. However, it is apparent that a greater or lesser proportion of the soil lead content may have originated from atmospheric sources, depending on location and indigenous lead content (Ratcliffe, 1981).

Lead may inadvertently be added to foods during processing. The practice of canning foods, for instance, may lead to a considerable increase in lead content, at times up to 2 or 3 times higher than those of products sold in glass or other containers (Ratcliffe, 1981). The tin coating (of cans) itself contains little lead, if any, but the solder used for the seam may contain up to 98% lead (FAO/WHO, 1972). It has been estimated that canning is responsible for two-thirds of the lead present in foods and most of that amount is derived from the solder in side-seam cans (Miller, Anderson and Falci, 1983). Since commercial baby foods may comprise the bulk of an infant's dietary intake in Western countries, the lead content of canned infant foods has been in focus for some time. Stringent regulations now exist in the united States and the United Kingdom on the lead concentrations of such foods (Harrison and Laxen, 1981; Ratcliffe, 1981).

Another possible commercial source of lead contribution to the food is from food wrappings. Watkins *et al* (1976), for example, had reported the presence of alarmingly high levels of lead in icecream bar wrappings (1400 mg/kg (ppm)) and bread wrappings (28,700 mg/kg (ppm)). The coloured portions of wrappers of some speciality foods (e.g. bakery confections, lollypops, chewing gum, candy, etc.) tested by Hankin, Heichel and Botsford (1974) were shown to contain lead ranging from 8 to 10,000 ppm, which could be a potential hazard to children.

Contamination of foods from housewares has aroused considerable concern. For instance, leadglazed ceramic cooking vessels and tableware have been known for some time to be able to release the metal into the food and beverages (Mahaffey, 1978). This leaching of lead from glazes depends on several factors, such as type of glaze, the firing temperature, the length of time the food or beverage remains in the vessel, and its acidity (Mahaffey, 1978; Ratcliffe, 1981). The problem appears to arise particularly in the case of products such as beer, wine, fruits or fruit juices, and foods preserved in vinegar (Ratcliffe, 1981). Aside from glazes, which is the most frequent sources of lead contamination of food from pottery, the clay used in pots in some parts of the world has been reported to be high in lead, making such unglazed pots potentially hazardous (Mahaffey, 1978). Vitreous enamelling, commonly used on a variety of kitchen ware, may also be a source of lead in foods and beverages (Ratcliffe, 1981).

2.2.1.2 Lead in Individual Foods

Lead has been found in virtually all foods, from both primitive and industrial societies, although concentrations are highly variable (IARC, 1980). Numerous reviews must have been written on lead content of foodstuffs. With the assumption that there is no gross contamination or adulteration of the food products, certain broad generalizations regarding the lead content of foods may be made (Mahaffey, 1978). Fruits and vegetables may contribute considerable amounts of lead to the diet. Foods of animal origin, i.e. muscle meats, unprocessed milk, and eggs are said to be relatively low in the metal. However, organ meats, particularly kidney, and to a lesser extent liver, have been observed to be substantially higher in lead concentrations than the muscle meats. Since lead is known to be mostly deposited in the bone, food products derived from it, e.g. bone meal, can be grossly contaminated with lead. Processed foods generally are expected to contain more lead. For instance the concentration of lead in processed cow's milk is higher than in human milk or in milk obtained directly from cows (WHO, 1977). However, certain highly processed foods, such as refined sugar and oils are said to be often low in lead and other trace elements (Mahaffey, 1978).

Reviewing results of studies carried out by the Ministry of Agriculture, Fishes and Food (MAFF) of the United Kingdom, and the Food and Drugs Administration (FDA) of the United States, Ratcliffe (1981) concluded that the lead content of the majority of foodstuffs is in the region of 0.1–0.2 μg/g fresh weight, with the exception of certain foodstuffs such as shellfish, condiments and certain dehydrated and concentrated foods and canned foods. Harrison and Laxen (1981) has tabulated the lead content of some foods surveyed and reported by the MAFF from 1972–1974. These authors further noted that with improved handling practices, the lead content of foods is generally declining in both Britain and the United States.

A brief mention will be made of the contribution of lead in beverages to human lead intake. In recent decades, the most severe lead contamination of beverages has occurred in illicitly produced whisky or "moonshine" (Mahaffey, 1978). Lead contamination also occurs in beverages purchased through regular commercial channels such as table wine, beer, cider and soft drinks, and could contribute significantly to lead intake, especially in populations consuming large amounts of these (Harrison and Laxen, 1981).

2.2.1.3 Lead in the Diet

Dietary lead intake may be estimated in three ways: (a) calculation, with the aid of tables of lead content of foodstuffs, based on estimates from food consumption surveys; (b) determination of lead content of food and beverages that duplicate an individual's diet; and (c) measurement of fecal lead excretion

(Mahaffey, 1978). Various estimations of lead in the diet have been made. Mahaffey (1978) has reviewed several studies in various countries, carried out before the 70s. Various studies in children, including some recent ones by Mahaffey were also reviewed. Ratcliffe (1981) has separately tabulated the estimates of daily oral intake of lead for adults and two-year old children reported by various investigators. Harrison and Laxen (1981) has reviewed and tabulated some of the more recent data, reported since 1970, for studies carried out mainly in the United States and United Kingdom, and a few in Finland and Canada. As to be expected, a high degree of variability in dietary lead intake was noted. These authors suggested that a typical intake probably lie in the range of 100–200 μg/day for adults and somewhat lower but not proportionately so, for children at 50–150 μg/day. Mean daily dietary lead intake as determined in the FDA Total Diet Studies Program has been summarised by Biddle (1982). Figures for infant, toddler and adult were tabulated for 6 years, commencing from 1975. There appears to be no clear trend to lead intake for the different years, although intakes in 1979 and 1980 seem to be higher than those in 1975 and 1976. These results for the United States were slightly lower than those presented by Harrison and Laxen (1978) (discussed above) for several countries.

2.2.2 Lead content of Malaysian foods

At this Institute, work is being carried out by the authors on the lead content of commonly consumed Malaysian foodstuffs (Siti M. Shahid, Tee and Chong, 1984). Samples of the different groups of foodstuffs were purchased from the markets and foodstalls in Kuala Lumpur and nearby areas. So far a total of some 30 different kinds of food commodities have been examined using both the colorimetric dithizone extraction and the organic extraction methods. The former was found to be rather elaborate and the dithizone reagent is easily oxidised especially when the iron content of the food is high. Hence, the latter method is preferred.

In the organic extraction method used, the food samples can be dry-ashed or digested by using sulphuric acid and hydrogen peroxide. In the latter instance, digestion need not be complete. Lead is extracted from acidic solutions (either the dissolved ashes or the residual solution after acid digestion) into xylene as its diethyl ammonium diethyldithiocarbamate chelate, and then determined by the use of atomic absorption spectrophotometry. Large amounts of iron and tin do not appear to interfere in the determination.The method also avoids most of the background problems and increases the sensitivity. Problems of pH adjustment do not arise with this technique and in favourable instances the determination can be carried out without preliminary ashing of the product (Roschnik, 1973).

Preliminary results obtained by the authors are presented below. These are

not to be taken to be representative, but serve to give an idea of the possible range of lead levels of some of the food items in the various foods groups. The findings are compared with those reported by investigators in the other local institutions, especially those of Chia (1983) of MARDI, as well as those from other countries.

2.2.2.1 Eggs

Fresh eggs were found to contain lead in the range of 0.47–0.48 ppm, well below the generally allowable limit of 2 ppm (e.g. NHMRC, 1981). The preserved eggs (*pidan*), with a lead level of 2.18 ppm, seem to exceed the limit. This high lead content is said to be attributable to the nature of the preservation process (Hou, 1981). It is to be noted that the samples analysed by Krinitz and Tepedino (1981) contained lead levels ranging from 5.5 to 30.7 ppm.

2.2.2.2 Fish, Meat and Poultry

Approximately fifty samples of commonly consumed fish and shellfish were analysed for lead content. The mean level of lead obtained was 1.66 ppm, slightly higher than the permissible limit proposed by the Ministry of Health Malaysia (1984) of 1 ppm. For meat and poultry, the mean lead level was 0.69 ppm. Results obtained for these groups are found to be similar to those reported by Chia (1983). Data obtained by the United States FDA on the lead content of milk, eggs, meat, fish and shellfish indicate that the mean levels in these unprocessed foods varied from only 0.02 to 0.4 ppm (Jelinek, 1982). This would appear to be lower than similar foodstuffs obtained by authors.

2.2.2.3 Cereal and Sugar Products

The levels in some cereal products studied ranged from 'not detected' to 1.86 ppm. The lead levels in both granulated sugar and *gula melaka* were found to be about 2 ppm, approximately 4 times higher than the proposed maximum permissible limit of 0.5 ppm for refined sugar (Ministry of Health Malaysia, 1984). This is in contrast to the finding of Mahaffey (1978) that refined sugar and oils are generally low in lead contamination.

2.2.2.4 Vegetables and Fruit

Vegetables and fruits studied at the Institute were shown to contain lead ranging from 'not detected' to 1.69 ppm. Permitted level for this food group is 4.0 ppm (NHMRC, 1981). Levels of lead in various vegetables and fruits examined by Chia (1983) were found to be in the range of 1.8–4.5 ppm and 0.1–1.7 ppm respectively. The samples of vegetables studied by Low and Lee

(1979) had lead levels ranging from 0.25 to 0.84 ppm.

A recent local report that should be of interest is the study of Lim, Domala Zakariya and Khoo (1983) which determined the lead concentrations of breast milk among urban and rural mothers. Mean lead levels of urban samples were found to be significantly higher than that from the rural areas. However the estimated daily lead intake of breast-fed infants in Malaysia was well below proposed tolerable levels.

The majority of the local foods thus far studied by the authors do not appear to contain lead in hazardously high levels. It must however be admitted that the samples analysed are insufficiently large. Work in the area is continuing. Aside from the studies of Chia (1983), there are no other large studies of lead content of local foodstuffs. It is hoped that other investigators, especially those from other parts of the country, could contribute towards our knowledge on the subject.

2.2.3 Other sources of ingested lead

The other sources of ingested lead contributing to total human exposure to lead will be briefly discussed. Dust and soil have been known for many years to contain high concentrations of lead. This lead can originate from several sources, e.g. flaking or washing of leaded paint from the exterior of dwellings, mobile and stationary source emissions and natural sources (Ratcliffe, 1981). If dust or soil in a child's environment contains high concentrations of lead, more of the metal can be expected to be present on the hands or objects that are handled and hence available for ingestion via mouthing activities of the child.

Lead-containing paint as a cause of lead poisoning has also been well documented. For instance in pica, a habit prevalent in certain cultural groups, a significant intake of lead could result if lead-based paint is ingested. The problem would be more serious in the presence of old paintwork and plaster with high concentrations of lead, particularly in old pre-war dilapidated housing where peeling and cracking of surfaces is common, affecting primarily the poorer communities (Ratcliffe, 1981). The lead content of paints and constituents used in the manufacture of toys, crayons and pencils are now restricted. Locally, examining 29 brands of pencils and 5 sets of coloured pencils, Low and Lee (1983) found that pencils made in Malaysia were generally low in lead, but two brands from Japan had high levels of acid extractable lead (> 1000 ppm).

Another source of exposure, especially to infants and young children, is lead in the ink used in coloured newsprint. Hankins and co-workers, for instance, found that lead in magazines ranged from 8 ppm for black and white pages to 3600 ppm for coloured ones (Hankin, Heichel and Botsford, 1973). The WHO (1977) report cited lead contents of between 1140–3170 mg/kg (ppm) for inks used in coloured magazines illustrations.These sources

would be of particular significance to children prone to pica or to the normal mouthing and licking habits of young children.

2.2.4 Lead in water

Drinking water generally contains a small amount of lead. This lead may be added to water from soils and rocks naturally containing lead, from industrial and vehicular sources of lead by aerial fallout or via soils, dusts from waste, and from the lead in pipes used to distribute potable water supplies (Ratcliffe, 1981). With regards to lead in pipes, various factors in turn may influence the lead content, such as the length of lead piping, the presence of lead soldering at pipe joints, the use of leadlined water storage tanks, and the length of time a given body of water remains in contact with the lead piping, as well as the chemical properties of the water itself (Mahaffey, 1978; Ratcliffe, 1981). Although far higher concentrations of lead in water do occur, under usual circumstances, most water supplies in the United States and the United Kingdom have been estimated to contain less than 10 μg Pb/litre. Unless lead concentrations of the water are unusually high, even persons drinking large amounts of water are exposed to relatively little lead from this source (Mahaffey, 1978). Harrison and Laxen (1981) have estimated that daily lead intake from water to be between 1 and 2000 μg of Pb, the values being usually < 20 μg/day

2.2.5 Lead in air

The question of the contribution of airborne lead to the human burden of lead has caused much controversies (Bethea and Bethea, 1975). The issue has drawn more emotional debate than any other aspect of lead in the environment (Nriagu, 1980). This section will not deal with this in detail; only a brief discussion of the possible sources of lead to the atmosphere will be presented.

Lead in the atmosphere is derived from a wide variety of natural and anthropogenic sources. According to the estimates of Nriagu (1979) for the worldwide emission of lead from these two sources for 1975, the release of lead from natural sources is said to be small (about 5%) when compared to technogenic emissions. More than half of the annual technogenic emissions in turn has been estimated to be from the combustion of lead-containing petroleum. In recent years, much concern has been raised regarding the emission of lead from vehicle exhausts as a result of the use of lead alkyl anti-knock additives in petrol. The other major contributors of airborne lead include various stationary sources, e.g. the production of steel and base metals, the mining and smelting of lead and the non-automative burning of fuel.

Nriagu (1980) has estimated that the current levels of lead in air at most rural and urban areas in the world are 50–4000 fold higher than the levels in pre-industrial atmosphere. There is said to be increasing concern that this would

contribute a health hazard to man, particularly to children. This airborne lead, likened to a primary source, could result in secondary and tertiary contamination of the environment. Hence, besides being directly inhaled by man, could contribute via fallout to the lead in soils and water, which in turn affects the lead concentration in the various foodchain (figure 1).

Locally, several investigators from the UPM have studied some aspects of atmospheric lead, resulting from automobile exhausts, in the Kuala Lumpur area. Considerable accumulation of lead was found in soil and grass adjacent to high traffic density areas (Low, Lee and Yusof Hj. Arshad, 1979). In many areas the concentrations of lead in grass were said to have reached the level considered harmful to livestock. In another study, these authors reported a higher concentration of lead in vegetables grown near the Kuala Lumpur – Ceras Highway, compared to those grown in a domestic garden of the UPM campus (Low and Lee, 1979). In a more recent study, these investigators observed that there was a definite distribution pattern for lead in mosses, depending on the traffic density of the areas. It was concluded that mosses are good indicators for lead pollution in the atmosphere (Lee *et al,* 1983).

2.3 Lead Metabolism

Having considered the various possible sources of exposure of man to lead it would be essential to next discuss how the body handles this contaminant: the absorption, distribution, retention and excretion of lead will be discussed. It would also be necessary to know the various indicators that may be used to measure such exposures or intoxication: these will also be briefly discussed in this section. It is of relevance to note a recent review by a local investigator on lead metabolism (Khoo, 1980).

2.3.1 Absorption

Inorganic lead compounds are absorbed via the lungs and gastrointestinal tract. However, organic acids (e.g. lead napthenate and lead stearate, such as may be present in some cosmetics) are, to some extent, also absorbed through the skin. Absorption through the intestinal tract is the most important route in non-occupational exposure, whilst absorption through the lungs is more common in occupational exposures (WHO, 1980).

Gastrointestinal absorption of lead is a relatively inefficient process and most of the lead ingested passes directly to the faeces (Harrison and Laxen, 1981). Using lead isotopes to trace the absorption of ingested lead, studies have revealed that absorption is highly variable. Investigators have reported the absorption of orally ingested lead ranging from 1 to 40%, although a figure of 5–10% has often been adopted (Grandjean, 1975; WHO, 1980; Biddle, 1982; Mahaffey, 1983). Absorption has been known to be dependent on the physical and chemi-

cal characteristics of the ingested lead and dietary composition (Ratcliffe, 1981). Studies have indicated that dietary factors such as low calcium, phosphorus, iron and protein content of the food increases gastrointestinal absorption (Mahaffey, 1980; WHO, 1980; Ratcliffe, 1981; Biddle, 1982; Barltrop, 1983). It is also known that infants and young children may absorb proportionately more lead from the gut than adults (WHO, 1980; Mahaffey, 1983). Specific mechanisms of lead absorption from the intestine remain largely unexplored and unexplained, although vitamin D has been suggested as an important mediator in the process (Rosen and Sorell, 1978).

The greater part of airborne lead is present principally as a fine aerosol of inorganic lead salts, which is readily inhaled into the alveolar region of the lung. Only a part of inhaled lead is deposited, the remainder being exhaled (Harrison and Laxen, 1981). The quantity of lead that is retained in the lung, the site of deposition and the amount subsequently absorbed depend on various factors such as: the concentration of particles in the atmosphere over time; the particle size, shape and density; the particle solubility in the epithelium and endothelium of the lung, and the rate and depth of respiration (Ratcliffe, 1981). About 30% of the lead inhaled may be absorbed through the lungs. The larger particles are deposited in the upper respiratory tract from where they are transported by the mucociliary escalator to the nasopharynx and swallowed (WHO, 1980).

2.3.2 Distribution, Retention and Excretion

The absorbed lead enters the blood stream and rapidly attaches itself to the red blood cells. There is a further rapid redistribution of the lead between blood, extracellular fluid and other storage sites, depending on the relative affinity of each tissue for lead, such that only about half of the freshly absorbed lead is found in the blood after only a few minutes (Harrison and Laxen, 1981).

The lead present in the body can be divided into two types: exchangeable fraction and the stable fraction. The former consists mainly of lead in blood and in soft tissues, especially liver and kidneys. The concentration of lead in blood is in equilibrium with that of lead in soft tissues. The stable fraction, which makes up 90% of the total body burden of lead, is present in the bones and teeth, and represents the result of long-term accumulation throughout life (WHO, 1980). Thus, although the greatest percentage of the total burden of lead is associated with bone, it is fairly insensitive to short-term changes in lead uptake which directly affects health. Hence, the organ and tissue systems of the body, with the exchangeable lead burdens, are of greater toxicological significance (Harrison and Laxen, 1981; Biddle, 1982).

Lead may be removed from the body by several routes, the most significant being through urinary excretion (75–80%); lesser losses occur via gastrointestinal secretion (e.g. bile) (about 15%). Other routes, such as perspiration, deciduous teeth, hair or nails, account for less than 8% (WHO, 1977).

2.4 Biological Indicators of Exposure

This section discusses briefly the various parameters that may be used to detect increased lead uptake in the body. The various indicators may be classed as: (a) those that measure lead directly, e.g. lead in blood, urine, hair or deciduous teeth; (b) tests that measure the metabolic (mainly haematologic) effects from the ingested lead, e.g. blood levels of zinc protoporphyrin, free erythrocyte protoporphyrin, delta-aminolevulinic acid dehydratase activity, urinary coproporphyrin, or urinary delta-aminolevulinic acid.

2.4.1 Concentration of Lead in Biological Media

After absorption, lead is transported to the organs via the bloodstream. It can therefore be assumed that the concentration of lead in circulating blood reflects the amount of lead which has entered the body. It is however to be borne in mind that blood lead level reflects a dynamic equilibrium between exposure (absorption), retention, release and elimination (as discussed above). If the body burden is large, its relative impact on the blood level is great, and vice versa (WHO, 1980). Nevertheless, under the steady state conditions prevailing in the general population, blood lead level is generally considered to be a useful indicator of current exposure (WHO, 1980; Hernberg, 1980; Biddle, 1982). Many studies have indicated that the average blood lead level in adults without occupational exposure is usually in the range of 100–250 μg/litre (WHO, 1977). For many years, the measurement of blood lead level has been the principal biological index of dose, primarily because it has been considered to provide the best practicable estimate of the biological active part of the body burden of lead. Furthermore, the collection of blood is relatively simple, harmless, non-invasive and unobjectionable and can be performed on large samples of the population (Ratcliffe, 1981). Hence, it is the common parameter of dose in the experimental and epidemiological investigation of the toxicological effects of lead.

Owing to some limitations of whole blood analysis, for example its vulnerability to methodologic errors (Hernberg, 1980), increasing attention has been paid to the use of other indices, either was alternative measures or co-indicators to supplement the use of blood lead levels (Ratcliffe, 1981). The measurement of lead content in deciduous teeth has been used as a relatively non-invasive measure of dose; this index in fact is a generally acceptable measure of past exposure (Ratcliffe, 1981). Measurement of lead in hair allows the estimation of exposure over a period of one to several months, but the method may not be reliable because external contamination usually interferes and the results may be accurate, especially in occupational exposures (WHO, 1980). The excretion of lead in urine after chelation (usually with calcium-EDTA) is considered to be a good indicator of the mobile fraction of the metal in the soft

tissues (WHO, 1980; Ratcliffe, 1981). However, the use of the chelation cannot be considered on a routine basis, for both practical and ethical reasons, but is useful as a diagnostic tool in clinical lead poisoning (Ratcliffe, 1981).

2.4.2 Haematologic Parameters as Indices

The toxic effects of lead on the haematological system will be discussed in a later section. It is clear from the various features of these effects, particularly those on heme biosynthesis, that the changes in both the activity of enzymes in the system and the production and excretion of intermediates in the biosynthesis pathway can be considered as auxiliary or substitute indices of lead exposure (as well as being indicators of effect) (Moore, Meredith and Goldberg, 1980). Delta-aminolevulinic acid dehydratase (ALAD) appears to be the index that most closely correlates with blood lead since it responds immediately to an increase in blood level and recovers approximately at the same rate as the decline in blood lead after exposure. The analysis is also said to be accurate, precise and straight forward (Ratcliffe, 1981). ALAD activity is said to be a highly sensitive index of both environmental and industrial lead exposure, suitable for screening of a broad population (Moore, Meredith and Goldberg, 1980).

Although erythrocyte protoporphyrin (as zinc protoporphyrin – ZPP) levels do not respond immediately to a change in exposure unlike ALAD, but because of the practical aspects of analysis, and due to its comparative sensitivity over a wide dynamic range of exposure (ALAD may not be a sufficiently sensitive indicator at high ranges of exposure), this has become a major co-indicator for screening purposes for childhood lead exposure as well as for occupational exposure in the United States (Ratcliffe, 1981). Recently, a relatively rapid and simple method of determining ZPP on a drop of undiluted blood with a small portable haematofluorimeter has been developed (Blumberg *et al,* 1977). This would probably make the test more expedient for the screening of an industrially exposed group of workers (Moore, Meredith and Goldberg, 1980).

2.5 Toxicological Aspects

The toxic potential of lead has been known to man since ancient times. Warnings about hazards associated with its use have been found dating as far back as the second century B.C. (Needleman, 1980). With the realization that lead is ubiquitous in the environment as a result of continuous emission from various sources, man's need for understanding the toxic effects of the metal has become even more urgent. During the past decade, it has become increasingly evident that lead produces a spectrum of adverse effects. Although occasional episodes of classical lead poisoning still occur, particularly in young children,

acute exposure is becoming a diminishing problem. Of greater concern is the possibility that continuous exposure to lower levels of lead, as a result of widespread environmental contamination, may result in adverse health effects (Posner, Damstra and Nriagu, 1978). These effects have been best documented for the nervous, haematopoietic and renal systems, although a number of other essential body functions have been known to be affected.

2.5.1 Special Risk Groups

Prior to dealing with the adverse health effects that lead may have to man, it would be useful to first identify the sub-groups of the population that would be most susceptible to these effects. Such groups may be physiologically more susceptible to a given dose of lead. They may warrant special consideration from the point of view of biological guidelines, of acceptable exposure and dose, biological monitoring programmes and control strategies (Ratcliffe, 1981). Knowledge of the various factors that may determine these special risk groups are not well understood, but available data suggest that at least age and nutritional status are important factors (Ratcliffe, 1981). Thus, the "critical" population groups have often been considered to be young children and pregnant women.

A number of factors are thought to be involved in determining the greater susceptibility of infants and young children to lead than adults. These have been discussed in detail by Ratcliffe (1981), Mahaffey (1981, 1983) and Biddle (1982); a summary of these are given here. These children are known to have a higher metabolic rate than adults, so that their intake of food and drinks, and hence lead, is greater relative to their body weight than that of an adult. The habit of oral explorations, sucking fingers, mouthing and chewing objects, natural activities of this age group, is thought to further increase the intake of lead over that of an adult. Even at comparable levels of lead intake, infants and young children are known to absorb appreciably greater proportions of the ingested lead than adults do; hence with the increased intake, the situation could be aggravated. Increased absorption of lead has also been known to be associated with nutritional inadequacy (see particularly Ratcliffe, 1981 and Mahaffey, 1981); malnutrition amongst young children is already well known to nutritionists. Aside from these considerations, there is the further knowledge that haematological and neurological effects (the latter affecting mainly the central nervous system) of lead occur at lower levels in children, making this group deserving of special attention.

The second sub-group of the population of concern is the pregnant women and her developing foetus. Various studies have demonstrated that placental transfer of lead from maternal to foetal blood can occur (e.g. reviews by Bell and Thomas, 1980; Ratcliffe, 1981). The blood brain barrier is poorly developed in

the embryo and neonate, and the developing neural tissue may be expected to be particularly vulnerable to any lead that may reach it (Ratcliffe, 1981). Besides, excessive lead exposure can indisputably increase the incidence of miscarriages, stillbriths, premature deliveries and reduced pregnancy rates (Ratcliffe, 1981).

Another special risk group is felt to deserve special attention is the workers in lead industries due to their potentially high exposure to lead (Mahaffey, 1981).

2.5.2 Clinical sequelae

A wide variety of signs and symptoms have been described for lead poisoning. Acute signs and symptoms may result from both short-term massive exposure and from long-term lead intake. After an initial stage of anorexia, symptoms of dyspepsia, and constipation, there is an attack of colic characterised by diffuse paraoxysmal abdominal pain. The skin is usually pale, the pulse slow, and the blood pressure may increase (Tsuchiya, 1979). The signs and symptoms in children are said to be somewhat different than in adults. For example, peripheral neuropathy is more common in adults, while encephalopathy is much more common in children (Posner, Damstra and Nriagu, 1978). Encephalopathy is the most severe form of lead poisoning. It typically develops over a few days or weeks with the onset of vomitting, ataxia, periods of alternative stupor and hyperirritability. Seizures or convulsions may occur and may proceed to a state of coma and death (Ratcliffe, 1981).

2.5.3 Neurobehavioral toxicity

It has long been recognized that lead is potentially toxic to the nervous system. By the turn of the century, neurological symptoms such as optical atrophy, tremors and wrist drop were already recognized as the consequence of excessive lead exposure in occupationally exposed adults. In children, on the other hand, it has been well known that the most advanced stage of acute "clinical" lead intoxication is encephalopathy, a syndrome of severe neurological symptoms which may culminate in coma and death (Ratcliffe, 1981). Neurobehavioral toxicity however, is a relatively new field of study. In recent years, there has been a growing concern, which has received a great deal of publicity, on the possibility that chronic, asymptomatic lead exposure may cause "minimal brain dysfunction", behaviour problems, and neurological impairment in children exposed to lead in utero and/or during early childhood (Posner, Damstra and Nriagu, 1978).

For many years, it was not clear that following the acute phase of lead intoxication, significant residual effects could remain. Byers and Lord (1943)

reported following 20 children who were assumed to have recovered from lead poisoning for several years. It was observed that 19 of these children were failing in school or were severely disordered in their behaviour. It is thought these investigators had raised for the first time the question of whether some idiopathic neuropsychologic deficit could result from lead intoxication (Needleman, 1980). Behavioral consequences, of all the hazards known to be associated with lead exposure, is often the most insidious. In many cases of childhood lead poisoning, delayed behavioral and/or intellectual effects of early lead exposure may not be recognized until the child enters school (Jason and Kellogg, 1980). It is also clear that such consequences are the most difficult to diagnose. However, with the advent of increasingly reliable techniques in behavioral toxicology, subtle neurobehavioral effects of lead are now better quantified (Jason and Kellogg, 1980). Some recent studies in the area are outlined below to illustrate the possible subtle, neurobehavioral effects that may be associated with chronic low-dose exposure to lead.

2.5.3.1 In Childhood Exposure

Several comprehensive recent reviews on the subject have been made available. The reviews of Jason and Kellogg (1980), Bornschein, Pearson and Reiter (1980) and Ratcliffe (1981) are of relevance. In the most recent one, Ratcliffe (1981) had summarised and tabulated 25 studies, commencing from the earlier study of Byers and Lord (1943) to recent studies such as that of Needleman *et al* (1979). The following account have been summarised mainly from these reviews.

Since lead encephalopathy was a well documented phenomenon, the earliest studies of behavioral and intellectual effects of lead focused on the question of whether there were long lasting sequelae in children hospitalized for lead poisoning, such as that reported by Byers and Lord (1943), and the later and larger study of Perlstein and Attala (1966). Later studies in the area, in the 1970s, had investigated behavioral changes in asymptomatic children with a history of lead exposure (e.g. the study of de la Burde and Choate (1975) of children eating plaster or paint during early childhood), or those with elevated blood or tissue (hair, teeth) lead levels (such as the studies of Albert *et al* (1977), Needleman *et al* (1979) and Winneke (1983). The last two mentioned studies are perhaps the most comprehensive investigations into the problem. These investigators had studied school-age children, using lead content of the deciduous teeth as a measure of long-term cumulative lead exposure during childhood. Various deficits in neuropsychological functions were observed for the lead-exposed group, compared with the control group, even after taking in consideration a number of non-lead variables known to be important to neuropsychological development.

Many of the studies in the early 70s had assumed that the method of

exposure to lead was ingestion of peeling paint- and/or plaster-containing lead, especially under poor housing conditions. With the realization that lead intoxication from this route may be decreasing (with the probable exception of the United States), later investigators had turned to examining children living in high lead environments. It is increasing being recognized that these children may be ingesting excess lead from such environments (Jason and Kellogg, 1980). The studies of Landrigen *et al* (1975a, b) in Texas, and Landsdown *et al* (1974) in London, of children living near smelters and the study of Ratcliffe (1977) of children residing close to a lead batter manufacturing plant in Manchester, are examples of such studies of community exposure.

Ratcliffe (1981) has pointed out that the various studies reported had differed markedly in design, criteria of does of 'test' and 'control' groups, sample size, criteria of matching of groups, types of tests employed, and type of population studied. The major problems of such studies are said to be in the classification of exposure, in ascertainment of bias, confounding with other variables, and insensitive measures of dependent variables (Needleman, 1980). Reviewers have noted that findings vary widely regarding the intellectual and behavioral deficits associated with increased lead exposure (needleman, 1980; Jason and Kellogg, 1980; Bornschein, Pearson and Reiter, 1980; Ratcliffe, 1981). Most of the differences in results are thought to be attributable to differences in study design and the selection of experimental and control subjects (Jason and Kellogg, 1980). Such retrospective epidemiological surveys have thus failed to establish unequivocal dose-effect and dose-response relationships between neurological dysfunction and lead, particularly for moderate level of exposure (Ratcliffe, 1981). Bornschein, Pearson and Reither (1980) and Winneke (1983) have emphasized the importance of prospective, longitudinal studies, since the cross-sectional, essentially retrospective approach adopted by most investigators could only provide suggestive evidence for lead being the causative factor for the observed neurobehavioral effects. Nevertheless, Jason and Kellogg (1980) felt that the overwhelming conclusion of investigators is that adverse behavioral and intellectual effects do occur with increased lead burdens in so-called asymptomatic children.

2.5.3.2 In Chronic Occupational of Exposure of Adults

The possible neurobehavioral effects of chronic exposure in adults has not attracted as great an attention as that in children. The chronic exposure of adults to lead occurs primarily in the workplace. Lead smelters (especially secondary smelters) and storage (lead-acid) battery manufacturing plants where lead casings are produced are among the most hazardous of working environments in terms of lead exposure (Jason and Kellogg, 1980). Examples of studies of workers in storage battery factories are those of Seppalainen and Hernberg (1972), Sep-

palainen *et al* (1975) in Helsinki, whilst Valcuikas *et al* (1978) had reported studies of secondary lead smelter workers in California. Distribances in behavioral and sensory functions of these workers were documented by these investigators.

The number of studies of occupational lead exposure are relatively small. Available data, however, are said to demonstrate that several neurobehavioral deficits occur in workers exposed to lead at levels which do not produce clinical symptoms (Jason and Kellogg, 1980).

A study on occupational exposure to inorganic lead at a battery manufacturing factory in Petaling Jaya, Peninsular Malaysia was recently reported by the Occupational Safety and Health Division of the Factories and Machinery Department of the Ministry of Labour and Manpower (Wan, Abu Bakar Jaafar and Abu Bakar Che' Man, 1976). Blood lead and urinary delta-aminolevulinic acid of 104 workers in the factory were found to be higher than that in a control group. Commonly observed early symptoms were lassitude, metallic taste in the mouth, fatigue and headache. The late symptoms reported were abdominal colic, diminished muscular ability or strength, obstipation and inability to concentrate. The study was recently extended to 8 factories, and covering 251 workers (Lim and Abu Bakar Che Man, 1983). Air sampling showed that a higher proportion of the smaller factories had lead-in-air levels exceedings 150 $\mu g/m^3$. In sharp contrast to the larger factories, a high percentage of the workers in these small, locally-owned companies had blood lead concentrations above the proposed limits of 70 $\mu g/100$ ml for males and 40 $\mu g/100$ ml for females. Three of these workers also exhibited positive signs or symptoms. It was concluded that there was an acute problem of environment lead exposure and excessive lead absorption in Malaysian battery workers, particularly those employed in the smaller factories. It was emphasized that there is an urgent need for education and legislative control to safeguard the health of workers in the lead industry in the country.

2.5.4 Anaemia of lead poisoning

The haematological effects of lead are said to be the only ones for which the biochemical mechanisms and dose-effect and dose-response relationships are well established (Ratcliffe, 1981). Three mechanisms are involved in the development of anaemia during lead poisoning (Moore, Meredith and Goldberg, 1980): (a) depression of heme biosynthesis; (b) disturbance of globin synthesis; and (c) haemolysis of red blood cells.

The effects of lead upon heme biosynthesis, which are said to have been long recognized, and upon the initial stages of heme degradation, have recently been reviewed by Moore, Meredith and Goldberg (1980). The principal effects are said to be upon the biosynthetic enzymes. Those inhibited are delta-aminolevulinic acid dehydratase, coproporphyrinogen oxidase and ferrochelatase, with

the first named the most affected. As a result of these changes, there is an increase in the production and excretion of the intermediates of the heme biosynthesis pathway, delta-aminolevulinic acid, coproporphyrin and protoporphyrin. These, together with delta-aminolevulinic acid dehydratase have been used as secondary indices of lead exposure.

Consistent with the observation that lead can affect protein synthesis, it can also affect the synthesis of globin both in vitro and in vivo (Ali and Quinlan, 1977). It interferes with amino acid incorporation and, when reticulocytes are incubates with lead, one finds disaggregation of polyribosomes (Moore, Meredith and Goldberg, 1980).

Lastly, lead also causes anaemia by shortening of the life-span of the erythrocyte. The exact mechanism whereby lead exerts this effect is not fully understood, although several possibilities have been suggested, including increased osmotic resistance and increased mechanical fragility (Ratcliffe, 1981).

2.5.5 Renal effects

Lead can produce both acute and chronic nephropathies. In both cases, it is the excretory mechanisms of the kidneys which are mainly affected (Biddle, 1982). The renal effects of acute lead toxicity include degeneration of the cell lining of the proximal tubules, varying degrees of cellular necrosis, and decreased re-absorption of amino acids, glucose, and phosphates. Clinically, severe acute lead nephropathy is manifested by aminoaciduria, glycosuria, and hypophosphatemia (Choie and Richter, 1980; Biddle, 1982). All these functional and morphological changes are reversible upon treatment with chelating agents such as EDTA, but only in cases of relatively short-term lead exposure (Chisolm and Leahy, 1962).

Long-term exposure to lead may give rise to the development of irreversible functional and morphological renal changes (Tsuchiya, 1979). Such changes in chronic lead nephropathy have not been well characterised (Choie and Richter, 1980), although there appear to be intense interstitial fibrosis, tubular atrophy and dilation, with involvement of glomeruli at a later stage (WHO, 1980). A consequence of such chronic renal damage may be saturnine gout, with increased excretion of uric acid in the urine (Tsuchiya, 1979).

2.6 CONTROL STRATEGIES

Several types of control strategies are needed for a multiple-source, multiple-route pollutant such as lead (Ratcliffe, 1981) :–

(1) Emission standards – emissions to air or water may be controlled by standards expressed as permissible mass of lead per unit time and per unit volume of air or water.

(2) Equipment or plant standards – these refer to, for example, design and operating standards for plants, designed to control emission of lead. Lead traps for vehicle exhausts would also be included in this category.
(3) Product specification standards – these include permissible levels of lead in, for example, glazes, paints, toys, cooking utensils, cans for foodstuffs, pipes and other products containing lead, including lead content of petrol. With regards to petrol or gasoline, several countries are aiming at lower lead levels in this fuel. In Malaysia, the maximum permissible limit of lead in petrol has been 0.84 g per litre since 1973 (SIRIM, 1973).
(4) 'Environmental' standards – these cover (i) maximum permissible levels of lead in food, beverages and water, based on the concept of a tolerable weekly or daily dose from ingested sources, such as that recommended by the Joint WHO/FAO Expert Committee on Food Additives (Joint FAO/WHO Expert Committee on Food Additives, 1972); (ii) maximum permissible concentration of lead in air to control atmospheric lead emissions from mobile and point sources; and (iii) guidelines or standards for lead in dust.
This section will only deal with the control of lead in food, beverages and water; details of other control strategies are found in, for example, Harrison and Laxen (1981), Ratcliffe (1981).

Regulatory agencies, such as the United States FDA, have initiated various programmes aimed at reducing lead exposure from foods and beverages to as low a level as possible, commensurate with the legal requirement not to "endanger the food supply" (Miller, Anderson and Falci, 1983). Primary focus of these programmes has been on reducing lead contamination in canned foods. In order to achieve the goals established , cooperation among regulatory agencies and the industry is clearly necessary. Miller, Anderson and Falci (1983) feel that many food processors are now converting, or will convert in the future, from the lead-soldered seam can to a welded seam can or to an aluminium or steel can without seams. In the near future, Malaysia will also have the welded seam can as the sole container for canning food as manufacturers are converting to this type of cans (Lim, 1984). This would, in due course, reduce the lead level by a substantial margin.

With better understanding of the special sensitivities of infants and young children towards lead toxicity, regulatory agencies have paid particular attention towards reducing lead levels in infant foods, of which a wide variety exists in Western countries. In the United States, the Infant Formula Council, an association of manufacturers of infant formula products, has also been set up to tackle the problem (Miles, 1982). Manufacturers of infant formula in the States have almost completely abandoned the lead-soldered 3-piece can; approximately 90% of liquid infant formulae in metal containers is now packaged in nonlead-soldered seam can (Miles, 1982).

Refering to another aspects of the problem, Muller and Schmidt (1983) pointed out that since infant formulae, available as a powder or as a concent-

rate, must be reconstituted with drinking water before they can be consumed, tap water supply could play an important role in the concentration of lead in the final product. Regulatory agencies have thus also been concerned with standards for lead in drinking water. Harrison and Laxen (1981) have tabulated the standards adopted by various countries.

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3 N – NITROSO COMPOUNDS

3.1 Introduction

The N-nitroso compounds, nitrosamines and nitrosamides have long been familiar with organic chemists (Magee and Swann, 1969). The hepatotoxicity of dimethylnitrosamine, the simplest aliphatic nitrosamine, in human was reported as early as in 1937 by Freund, who described clinical and autopsy findings of two chemists accidentally poisoned with this compound, then used as an industrial solvent. It was thus thought that exposure to nitroso compounds was confined to small groups of chemists or workers who were closely associated with these chemicals. In recent years, however, there has been considerable interest in the study of N-nitroso compounds.

Currrent interest in the nitroso compounds arose largely from the work of Barnes and Magee (1954) who demonstrated that dimethylnitrosamine is acutely toxic to experimental animals and subsequently, that it is also carcinogenic to these animals (Magee and Barnes, 1956). Another observation that had accelerated research on these compounds, leading to the currently available voluminous literature on the subject was the observation in Norway of severe liver disease developing in ruminants fed a diet containing fish-meal preserved with nitrite, and the identification of dimethylnitrosamine as the toxic principle in such feedstuff in the mid 1960s. It then became apparent that some of these nitroso compounds could exist in foods and feeds. Widespread efforts to detect the presence of these compounds in various human foods were carried out. Attention was soon directed towards other possible sources of contamination, such as the air, water supply and working environment. Studies on the modes of formation of nitrosamines had led to a search for the occurrence of precursors to the nitroso compounds, the nitrates, nitrites and amines. It was soon clear that these occur far more extensively and at greater concentrations than the preformed nitrosamines, and can lead to the formation of nitrosamines and nitrosamides in the air, water, soil, food, and *in vivo* in the human body, such as the stomach.

Todate, some 130 N-nitroso compounds have been examined for carcinogenicity and at least 120 of them have been found to be strong carcinogens to many animal species, including subhuman primates. So far, no animal species tested has been found to be resistant to the carcinogenic effect. These observations have led investigators to feel that it is difficult not to regard the human as a susceptible species as well. Various epidemiologic studies have thus been carried out to investigate the possible role that the nitroso compounds and their precursors could play in the aetiology of some human cancers. Although no direct evidences have thus far emerges from such studies, investigators feel

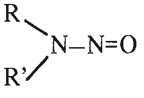
that available evidences suggest that the hypothesis should not be abandoned, and further work is certainly warranted. Meanwhile, steps have been taken by some authorities to reduce the contamination levels of nitroso compounds in our foods and drinks, including and especially the nitrates and nitrites.

In Malaysia, there has been few studies on the nitroso compounds. The levels of nitrosamines and their precursors in our foods and environment remain largely unknown. The few publications available will be cited in this review. It is hoped that this review will inject some interest and action amongst local investigators. With an understanding of the occurrence and formation of such compounds in our environment, we may be able to better understand the aetiology of some of the cancers occurring amongst Malaysians.

3.2 Chemistry

3.2.1 Structure

The general formula of nitroso compounds may be described as :–



They may be divided into two groups. In the nitrosomines, R and R' can be either alkyl or aryl groups or, in some cases, alicyclic. For certain cyclic nitrosamines, R and R' may be replaced by a cyclic ring as in the case of nitrosoazetidine and nitrosopyrrolidine (Sen, 1974). The nitrosamides usually have one alkyl residue R, and an acyl residue as R' (Preussman, 1983).

A convenient classification of this diverse class of N-nitroso compounds is given in Table 1 (adapted from Shank and Magee, 1981). Examples of each group is given in the Table, The simplest members of the nitrosamines are dimethylnitrosamine and diethylnitrosamine. Due to their simplicity in structure and easy availability, these have been widely used for various studies by investigators (Sen, 1974).

Based on physical properties of these compounds, and with particular reference to the analytical procedures applicable to each class, Fan *et al* (1978) have divided the N-nitroso compounds into four major, overlapping categories, as indicated in Figure 3.1.

3.2.2 General properties

The physical properties of nitrosamines vary widely depending on the nature and size of the substituent groups, R and R' (Sen, 1974; Scanlan, 1975). The following general description has been summarised from Crosby and Sawyer (1976) and Scanlan (1975). The simple aliphatic nitrosamines, e.g. dimethyl-

Table 1. Classification of N-Nitroso Compounds
(Retabulated from Shank and Magee, 1981)

1. Nitrosamines

A. Methylnitrosamines, $CH_3N(NO)-R$

e.g. dimethylnitrosamine

$CH_3N(NO)-CH_3$

B. Ethylnitrosamine, $C_2H_5N(NO)-R$

e.g. methoxymethyl-ethylnitrosamine

$C_2H_5N(NO)-CH_2-O-CH_3$

C. Propylnitrosamines, $C_3H_7N(NO)-R$

e.g. di–n–propylnitrosamine

$C_3H_7N(NO)-C_3H_7$

D. Butylnitrosamines, $C_4H_7N(NO)-R$

e.g. di–n–butylnitrosamine

$C_4H_9N(NO)-C_4H_9$

E. Other Aliphatic Nitrosamines

e.g. di–n–pentylnitrosamine

$C_5H_{11}N(NO)-C_5H_{11}$

diacetonitrilenitrosamine

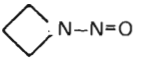
$NC-CH_2N(NO)-CH_2CN$

nitrósotrimethylhydrazine

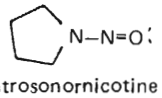
$(CH_3)_2-N-N(NO)CH_3$

F. Cyclic Nitrosamines R–N–N=O

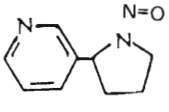
e.g. nitrosoazetidine



nitrosopyrrolidine



nitrosonornicotine



ii. Nitrosamides

$R-N(NO)CO-R'$

e.g. methylnitrosacetamide

$CH_3N(NO)CO-COCH_3$

methylnitrosourea

$CH_3N(NO)CONH_2$

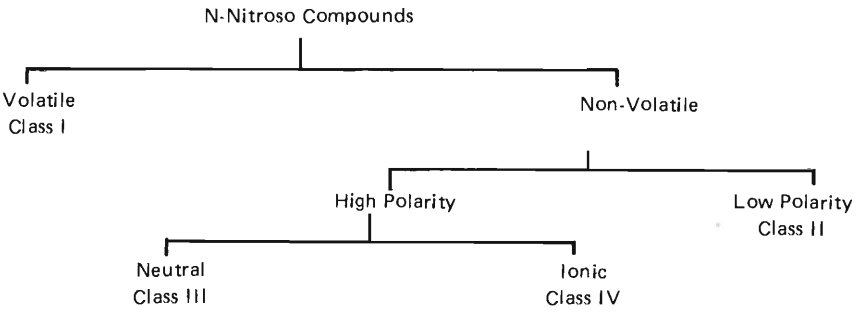


Fig. 3.1. Classification of N-Nitroso Compounds Into Four Broad, Overlapping Classes (Source: Fan *et al*, 1978)

nitrosamine, are yellow or yellow-green nonhydroscopic liquids which boil without decomposition, the boiling points lying between 150° and 220°C. They are partially soluble in water, the degree of solubility varying according to molecular weights, and they are readily soluble in organic solvents. On the other hand, the simple aromatic nitrosamines, such as methylphenylnitrosamine, are low-melting solids or yellowish oils of a characteristic "somewhat nutty odour". They are insoluble in water and can be distilled under reduced pressure, although at atmospheric pressure, they undergo decomposition. Nitrosamines are generally considered a more stable class of compounds than the nitrosamides. Nitrosamines are also known to be sensitive to UV radiation which splits the nitroso group.

A more detailed discussion of the chemistry of these compounds, including IR, UV, NMR and MS spectra are given in Crosby and Sawyer (1976). These authors have also discussed the various reactions that the nitrosamines may undergo, including complex formation, reaction with inorganic acids, reduction to hydrazines and to secondary amines, oxidation and nitration, cyclization to sydnones, and photochemical reactions. Details of chemical and physical data of individual nitroso compounds have been described in IARC (1978).

3.3 FORMATION OF NITROSAMINES

3.3.1 The nitrosation process

Synthesis of the aliphatic nitrosamine, diethylnitrosamine, was said to have been carried out at the end of the 19th century by the reaction of diethylamine hydrochloride with sodium nitrite (Crosby and Sawyer, 1976). Numerous investigations have since been carried out. It is now known that nitrosamines may be easily prepared by the action of nitrous acids on the corresponding

secondary amines. For example, dimethylnitrosamine is prepared by heating a mixture of equimolar quantities of dimethylamine hydrochloride and sodium nitrite in dilute hydrochloric acid. Similarly, secondary aromatic amines produce the corresponding nitrosamines after treatment with nitrous acid (Sen, 1974). In acidic solutions of nitrite and secondary amines, the following reactions have been suggested (Mirvish, 1975; Crosby and Sawyer, 1976; Shank, 1981a):-

(1). conversion of nitrite to nitrous acid:

$$NO_2^- + \overset{+}{H} \longrightarrow HNO_2$$

(2). conversion of nitrous acid to nitrous anhydride:

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O$$

(3). nitrosation of the unionized (unprotonated) amine:

$$\begin{matrix} R \\ R_1 \end{matrix}\!\!>\!\!NH + N_2O_3 \longrightarrow \!\!>\!\!NNO + \begin{matrix} R \\ HNO2 \\ R' \end{matrix}$$

The rate of the reaction is proportional to the nitrous anhydride concentration, and thus proportional to the square of nitrous acid concentration, hence the square of the nitrite concentration (Mirvish, 1970, 1975). Besides nitrous anhydride, other nitrosating agents including nitrosyl halide (e.g. NOCI), nitrous acidium ion ($H_2NO_2^+$) and nitrosyl thiocyanate (ON–NCS) (Mirvish, 1975).

Since the nitrous anhydride reacts on the unionized form of the amine, and because weakly basic amines (e.g. morpholine) ionize less readily than do strongly basic amines (e.g. dimethylamine), the nitrosation process has been known to occur more readily with amines of weak basicity and more slowly with strong basic amines (Shank, 1981a). It appears that nitrosation of the secondary and tertiary amines occur when the amine and nitrite are present in a solution of pH between 1 and 7, with maximum rates occurring between pH 3.0 and 3.4 for most amines (Mirvish, 1975; Shank, 1981a). For further details of the kinetics of the formation of nitrosamines, the various studies of Mirvish and coworkers may be refered to (Mirvish, 1970, 1975, 1977; Mirvish *et al*, 1973).

3.3.2 Formation in foods

Nitrate, nitrite and amines occur widely in many foods, water supplies and soil; given the proper conditions, for example during storage and food preparation, nitrosamines may be formed spontaneously via the reactions outlined above. Certain common vegetables have been found to contain high concentra-

tions of nitrate. Some are important contributors of nitrate ingestion because of their frequent consumption. Fresh vegetables generally contain little nitrite. However, upon storage, nitrates in vegetables may be converted to nitrites. Nitrates and nitrites are widely used for curing meats and fish. Nitrate is also present in water supplies, sometimes in unusually high concentrations. On the other hand, a wide variety of nitrosatable amines are present in the environment including many constituents of foods themselves and food additive. The occurrence of these precursors, the nitrate, nitrite and nitrosatable amines, are therefore of importance in determining the formation of nitrosamines in our foods and drink *in vitro*; these are further discussed in a subsequent section. In addition, the precursors are important in the formation of nitrosamines *in vivo*.

3.3.3 Formation *in vivo*

Prior to discussing the formation of nitrosamines *in vivo*, it is necessary to consider yet another source of the nitrites, i.e. the human saliva. It has been shown that nitrite is present in normal human saliva, and this is thought to have originated from the reduction of ingested nitrate by the oral microflora (Tannenbaum *et al*, 1974). This nitrite formation is dependent on the nitrate present, which in turn depends on the amount of nitrate ingested (Spiegelhalder, Eisenbrand and Preussmann, 1976; Tannenbaum, Weisman and Fett, 1976; Tannenbaum *et al,. 1977).* Subsequently, Tannenbaum *et al* (1978) had suggested that some of the dietary nitrite and nitrate could originate from *de novo* synthesis in the human intestine by heterotrophic nitrification of ammonia or organic nitrogen compounds. White (1975) had estimated that up to 30 mg of nitrate and 9 mg of nitrite could be found in the saliva over a 24-hour period. It woud appear therefore that saliva could be a major source of ingested nitrite (Shank, 1981a).

Studies by Sanders and co-workers (German publications cited by Sen, 1980 and Shank, 1981a) were said to have demonstrated the *in vivo* nitrosation of secondary amines by ingested nitrite (see also a review of this in Sander, 1973). The favoured site in the body for the reaction appear to be the stomach, where the acidic pH is optimum for the reaction (pH 3.4) (Mirvish, 1975; Lijinsky and Taylor, 1977; Shank, 1981a). Since nitrosation of amines is a function of the square of the nitrite concentration, it is felt that it is the concentration of nitrite in the stomach at any one time, not the total amount of nitrite ingested over a day, which is critical to the amount of nitrosamine that may be formed (Linjinsky and Taylor, 1977; Shank, 1981a). It was thus suggested that the importance of salivary nitrite in such reactions remains to be shown, since the concentration of saliva-derived nitrite at any one time in the gastric juice is small, especially when compared to nitrite concentrations resulting from ingestion of certain cured meats and from vegetables. Recently

Fine *et al* (1977) reported, for the first time, that nitrosamines could be formed *in vivo* in man following ingestion of conventional foodstuffs (a midday meal consisting of bacon, spinach and tomato sandwich and beer).

Although the stomach appears to be the favoured site for the reaction, it has been suggested that considerations should also be given to the reaction occurring in the mouth, oesophagus, intestinal tract, bladder and urinogenital tract (Tannenbaum *et al*, 1977). This was thought to be due to the fact that under normal or pathological conditions, these sites also contain bacteria which could reduce nitrate to nitrite to enable the nitrosation reaction to proceed.

3.3.4 Catalysts and inhibitors of nitrosamine formation

As mentioned earlier, the formation of nitrosamines is dependent on a variety of factors such as (1) concentrations of the reactants, (2) basicity of the amines, (3) pH and temperature. It is also known to be influenced by the presence or absence of other compounds which may act to enhance or inhibit the reaction.

Generally, weak anions such as thiocyanates, chlorides, bromides or iodide ions may act as strong catalysts to the nitrosation reaction (Boyland, 1972; Fan and Tannenbaum, 1973). Thiocyanate (NCS^-) has been shown to be effective in increasing the rate of nitrosation of morpholine, sarcosine, N–methylaniline, aminopyrine and other amines (Mirvish *et al*, 1973; Fan and Tannenbaum, 1973). The catalysis is said to take place by the formation of the nitrosating species ON. NCS (Shank, 1981a), different from the usual nitrous anhydride (N_2O_3). This catalysed reaction has been shown to be proportional to the nitrite concentration, unlike the reaction with N_2O_3, which (as has been discussed in the previous section) is proportional to the square of the nitrite concentration (Fan and Tannenbaum, 1973). Thus the thiocyanate catalysed reaction is thought to be especially important under conditions of low nitrite concentration (Shank, 1981a). Furthermore, since the thiocyanate ion is a normal constituent of human saliva (especially amongst smokers), its presence in the human stomach may markedly increase the *in vivo* formation of nitrosamines (Boyland, 1972; Boyland and Walker, 1974).

The presence of compounds which destroy nitrite before this can react with the amine can act as inhibitors of nitrosamine formation (Sen, 1980; Shank, 1981a). Various compounds such as ascorbic acid, ascorbyl palmitate, glutathione, cysteine, propyl gallate, gallic acid, tannins and alpha-tocopherol have been shown to be able to inhibit the formation of nitrosamines both in foods as well as *in vivo* in experimental animals (Bogovski *et al*, 1972; Mirvish *et al*, 1972; Greenberg, 1973. Sander, 1973; Fiddler *et al*, 1973; Sen and Donaldson, 1974; Ivankovic *et al*, 1974; Mirvish, 1975; Sen *et al*, 1976; Mergens *et al*, 1978). It is thought that the incorporation of these chemicals in foods or in

drugs, as part of the formulation, may be an effective means of minimizing human exposure to nitrosamines (Sen, 1980).

3.4 Occurrence of Nitroso Compounds In The Environment

3.4.1 Occurrence in foods and foodstuffs

World wide attention on the possible occurrence of carcinogenic N–nitroso compounds in foods and feeds seems to have started with observations in Norway of outbreaks of liver disease developing in ruminants fed a diet containing fishmeal prepared from sodium nitrite-preserved herring and the identification of dimethylnitrosamine as the toxic principle involved (Hansen, 1964; Koppang, 1964; Koppang *et al*, 1964; Sakshaug *et al*, 1965). The presence of other nitroso compounds in the fish meal had also been suggested, but they were not identified. This observation has led to widespread efforts to evalute levels of N–nitroso compounds in foods destined for human and animal consumption, especially those processed with sodium nitrite (Crosby and Sawyer, 1976; IARC, 1978a; Shank, 1981a).

Subsequently, many investigators began to report on the presence of nitrosamines in various commodities, with particular attention being paid to meat products. The occurrence of diethylnitrosamine in wheat, milk and cheese were reported by Hedler and Marquardt (1968) (using TLC to isolate the suspect compound and UV light to degrade the compound to "nitrite spots"; the presence of the nitrosamine was said to have been confirmed by GC). Ender and Ceh (1968) had reported the presence of dimethylnitrosamine in smoked fish, smoked meats and certain mushrooms (using the hydrazine method). The presence of diethylnitrosamine in spinach stored under conditions of high nitrite and hydrogen ion concentrations was observed by Keybets, Groot and Keller (1970). The occurrence of dimethylnitrosamine in the juice of the fruit of a solanaceous bush used in the preparation of milk curds by the Transkeian Bantus of South Africa was documented by DuPleissis, Nunn and Roach (1969) (employing TLC, GC, NMR and IR spectrophotometric methods). In another report from Africa, McGlashan, Walters and McLean (1968) had reported the occurrence of dimethynitrosamine in samples of distilled alcoholic beverage prepared by fermenting corn husks and sugar (methods used in the analysis included TLC and polarography).

Wasserman and Fiddler (1975), Crosby and Sawyer (1976), and Shank (1981a) however felt that some of these ealier studies (prior to 1970) gave rather unreliable results since the techniques used, such as polarography, gas chromatography with flame ionization detectors, and colour reactions, are now felt to be insufficiently specific to reliably demonstrate the presence of nitroso compounds. Moreover, many of the early methods had a limit of detection above the levels of nitrosamines now thought to be present in foods. These

authors felt that the use of selective gas chromatography detectors and improved isolation methodology, combined with confirmation of the presence and structure of nitrosamines, e.g. by mass spectrometry (MS) and thermal energy analyzer (TEA) in recent reports would provide greater assurance in the findings. Nevertheless, as pointed out by Shank (1981a), these earlier reports had led to a serious examination of the necessity of using sodium nitrite as a food additive.

It should be pointed out at this juncture that the analysis of nitroso compounds has been a particularly problematical area, and much research has been carried out. The International Agency for Research on Cancer (IARC), since 1968, had given high priority to the development and standardization of reliable, sensitive and specific analytical methods for the detection and quantitation of nitroso compounds in the environment (Bogovski, 1975). From thence on, the Agency has embarked on and coordinated in numerous studies and various activities in this area. These have been documented in various IARC publications (IARC, 1972, 1975, 1976, 1978b) and especially in IARC (1978c).

Shank (1981a) has given a convenient compilation and tabulation of the various reported occurrences of N–nitroso compounds in human foods and foodstuffs. A similar table has also been reported by Sen (1980). The levels of nitrosamines detected are usually very low, and in many cases, they were detected in only a small percentage of the samples tested. The major nitrosamines detected were dimethylnitrosamine, diethylnitrosamine, nitrosopyrrolidine and nitrosopiperidine, all of which are known to be potent carcinogens (Sen, 1980). Studies from these reviews and other reports are cited in the following paragraphs.

3.4.1.1 Cured Meat Products

In recent years, bacon has been extensively studied. One of the earliest reports after the 70s was the study of Crosby *et al* (1972) who observed after that nitrosopyrrolidine may be formed during frying; levels of up to 40 μg/kg (ppb) were found in some of the 20 samples examined. In fact, of all the cured meats tested so far, cooked bacon appears to be one item which has consistently been shown to contain fairly high levels of nitrosamines, mainly nitrosopyrrolidine (Sen, 1980). All the 8 samples of bacon analysed by Fazio *et al* (1973) were found to contain this nitrosamine in amounts ranging from 10–108 μg/kg. All the 50 samples tested by Gough *et al* (1977) and Gough, Webb and Coleman (1978) were found to contain nitrosopyrrolidine. Levels encountered had ranged from 1–20 μg/kg, with occasional samples going up to 200 μg/kg. The fatty portion of the bacon seems to contain more of this nitrosamine than the lean part (Fazio *et al*, 1973; Fiddler *et al*, 1974). It appears that uncooked bacon seldom contain this nitrosamine (Fazio *et al*, 1973; Sen *et al*, 1974), the levels increasing significantly as grilling temperature is raised (Telling *et al*, 1975), and maximal yields of the nitrosamine being obtained when frying

temperature approaches 400°C, regardless of the duration of frying (Fiddler *et al*, 1973). The fumes produced during the frying have also been shown to contain the nitrosamine; approximately 50% of the total nitrosopyrrolidine produced during cooking is volatilized into the fumes (Sen, Seaman and Miles, 1976). The precursors for nitrosopyrrolidine formation in bacon during frying have not been conclusively demonstrated although current evidence appear to suggest that both free proline and nitrosoproline, formed during the curing process, are the most likely (Shank, 1981a). Dimethylnitrosamine has also been found in cooked bacon, but at lower concentrations than those for nitrosopyrrolidine (Crosby *et al*, 1972; Sen *et al*, 1973; Gough *et al*, 1977; Gough, Webb and Coleman, 1978). Lesser amounts of diethylnitrosamine and nitrosopiperidine have also been reported in bacon (Shank, 1981a).

The reviews of Shank (1981a) and Sen (1980) revealed that a wide variety of other cured meats and sausages have been reported to contain nitrosamines. Nitrosopyrrolidine appear to occur at the highest concentrations, whilst dimethylnitrosamine, which is the most frequently encountered, usually occurs at lower concentrations. Diethylnitrosamine is found occasionally, and N–nitroso-di-n-butylamine and nitrosopiperidine appear to be found infrequently.

3.4.1.2 Other Processed Foods

Several other types of processed foods have been examined and reported to contain nitrosamines. Processed (fried, salted and smoked) fish appear to be the most frequently contaminated, with dimethylnitrosamine the most often encountered. The relatively high frequency at which this nitrosamine occurs is thought to be due to the presence of the free amines, dimethylamine and trimethylamine, compounds which contribute to the "fishy" smell of fishes (Shank, 1981a). The likelihood of nitrosamine formation is especially greater in the marine fish than in the fresh water fish, mainly because the former contains much higher concentrations of amines than the latter (Sen, 1980). Fong and Chan (1973) had reported fairly high levels of dimethylnitrosamine in a Chinese marine salted fish, which is considered a favourite dish along the southeast coast of China and many Southeast Asian countries. The replacement of crude salt (which contains nitrate as an impurity) with a purer grade of salt, and the addition of benzoic acid as a preservative was reported to have markedly reduced the formation of nitrosamines in these fishes (Fong and Chan, 1976). The problem of nitrosamines in fish could be of particular significance in this region since this is an important item of our diets.

Various cheeses too have been reported to contain nitrosamines. However, such reports after the 1970s are few; the most prominent being the studies of Crosby *et al* (1972) and Gough *et al* (1977). In fact, the latter report contained results of analysis of some 500 samples of various foodstuffs. The levels of nitrosamines in most of the foods were however less than 1 μg/kg, except

for cured meats and fish, and cheese (Gough *et al*, 1977).

The report of Fong and Chan (1977) of dimethylnitrosamine and nitrosopyrrolidine in some commonly consumed Chinese foodstuffs in Hong Kong (including dried shrimps, shrimp sauce and paste, oyster sauce, fish sauce, Chinese sausages and dried squid) could be of interest. Low levels of these nitrosamines were said to have been found. The nitrate and nitrite contents of these foods were also tabulated by these authors. Another report that could be of interest is that of Gough and Goodhead (1975) who studied the nitrosamines content of various spice premixes.

3.4.2 Other environmental sources of nitroso compounds

Human exposure to nitroso compounds was initially thought to be confined to a few microgram per day of dimethylnitrosamine and nitrosopyrrolidine in nitrite preserved foods such as those described above. Similar amounts of nitrosonornicotine in tobacco smoke were subsequently reported (Hoffman *et al*, 1976; Hecht *et al*, 1978). It was soon realized that man's exposure to nitroso compounds is much wider than these when nitrosamines were reported to be found in the air, soil, water, pesticides, cutting fluids, in comestics, and processed tobacco. It is becoming increasingly apparent that N–nitroso compounds are ubiquitous in the environment, particularly in the many chemical, agricultural and consumer products which characterise a modern industrial society (Fine, 1978). A more detailed review of such occurrences has been given by Shank (1981a). It has been emphasized that the relative risk of each of these items should be assessed in their proper perspectives (Sen, 1980).

3.5 Precursors Of Nitrosamine Formation

It is clear that man's exposure to nitroso compounds could be from nitrosamines and nitrosamides preformed in foods, the occurrence of which has been discussed in the previous section. Another important source of nitrosamines is the ingested precursors. These occur widely in the foods and drinks and, as discussed above, when ingested, could be used for the formation of nitrosamines in the body, such as the stomach. The nitrosamines so formed, according to Shank (1981c), appear to be the major source of human exposure to nitroso compounds; occupational exposure to these compounds rarely occur, and where they do occur, they could be controlled or avoided altogether. A consideration of these precursors, nitrates, nitrites and amines, are therefore of importance.

3.5.1 Nitrites and nitrates in meat

For thousands of years, man has learnt to preserve his food, particularly the more perishable ones, such as fish and meat. Lueck (1980) has given an interesting account of man's need for food preservation and the history of chemical food preservation. Smoking and salting were amongst the earliest methods used for preventing the spoilage of food by microorganisms. Smoking, in addition to reducing the moisture content of food, may also have a bacteriostatic action by virtue of substances such as phenols and aldehydes present in the smoke; changes of flavour have also been observed (Crosby and Sawyer, 1976). The curing of meat with nitrate containing salts has been practised for a long time. The historical background of the use of nitrates and nitrites in the curing of meat reviewed by Binkerd and Kolari (1975) should make interesting reading. In addition to its antibacterial action, the process has been known to enhance the palatibility and appearance of meats (Bonnett and Martin, 1976; Crosby and Sawyer, 1976).

It has been well known that upon cooking of fresh meat, its colour changes to brown. With salted meat however, it has been observed for some time that red patches were formed on the surface upon cooking. These patches were thought to be caused by nitrates present in the curing salt as impurities and hence, were subsequently deliberately added so as to obtain a greater uniformity of colour (Crosby and Sawyer, 1976). Nitrates has been used in food preservation for centuries (Binkerd and Kolari, 1975), although no one knows for certain who first employed this chemical for food preservation (Lueck, 1980). Only in the early 1900s was it determined that, in fact, nitrites were the agent responsible for the development of the red colour in cured meat products (Shank, 1981a), the nitrites being formed from nitrate by bacterial or enzymatic reduction (Crosby and Sawyer, 1976). Nitrates and nitrites are now widely used for curing many varieties of meat products. In the United States, it has been estimated that some 20% of all meats sold has been treated with nitrite (Lijinsky and Taylor, 1977). In addition to the generating of the characteristic pink colour of cured meats, nitrites also imparts a characteristic flavour to the product. In modern curing methods (Crosby and Sawyer, 1976), the process has been speeded up from several weeks to a few days, such as by using the direct multineedle injection into the vascular system of the carcass. In even more rapid curing methods, slices of meat are passed through a suitable curing solution, and the process is completed in only a few minutes.

The chemistry of this complex curing process has been discussed in some detail in Bonnett and Martin (1976). Essentially, it involves the interaction of nitrite with myoglobin under reducing conditions to form nitrosylmyoglobin, which upon cooking becomes denatured. It is these nitrosylmyoglobin pigments which give rise to the characteristic pink/red colour seen in cured meats.

It was not until the 1940s that the bacteriostatic property of nitrates was actively studied (tarr, 1941), and even then, the studies did not focus specifically on the inhibition of botulinum toxin production (Shank, 1981a). It is now clear that nitrite is added to foods to control the growth of *Clostridium botulinum* and consequential botulinum toxin production where subsequent heat treatment does not affect total sterilization and destruction of the toxin. The precise mode of the inhibition is not fully understood, but a number of interdependent factors are thought to be involved, such as concentration of nitrite and of salt, pH, degree of heat treatment, and number of spores initially present (Crosby and Sawyer, 1976). Various recent studies have been carried out to determine the actual levels of residual nitrite needed to confer protection from botulinum poisoning associated with eating vacuum packed meats (Christiansen *et al*, 1973; Hustad *et al*, 1973; Grever, 1973).

Botulism is one of the most serious forms of food poisoning known to man, refered to by Lamanna (1959) as "the most poisonous poison". It can occur in a variety of processed foods when the packing technique is not adequate (Shank, 1981a). Although outbreaks of botulinum poisoning have been relatively rare, several fatalities have been reported (Crosby and Sawyer, 1976). Hence, the use of nitrite as an additive to certain foods seems justified. On the other hand, investigators such as Shank (1981a) have emphasized that this benefit must be weighed with the evidence that the formation of carcinogenic nitrosamines in foods are almost totally related to the use of nitrite as a food additive.

3.5.2 Nitrates and nitrites in vegetables

Clearly, concentrations of nitrates and nitrites in foods vary a great deal and would depend upon the species, the climatic and soil conditions in which they were grown, and the handling procedures used, including during storage (Shank, 1981a). Some common vegetables such as cabbage, cauliflower, carrot, celery, lettuce, radish, beets and spinach have been shown to contain large amounts of nitrates (Sen, 1980). Spinach, celery and beets, for example, have been reported to contain, respectively, 2760, 2340 and 1860 ppm (mg/kg) nitrate (from data compiled by White, 1975). The increasing use of nitrogen fertilizers has been thought to have contributed to an increase in nitrate in vegetables, although nitrite content appeared to be unaffected (Lee *et al*, 1971). Fresh vegetables are said to be usually low in nitrite concentration (Sen, 1980; Shank, 1981a). Upon storage, nitrates are thought to be reduced to nitrite, as has been observed in the case of spinach (Phillips, 1968), especially under improper handling procedures for spinach and beet (Heisler *et al*, 1974).

White (1975) had estimated the average daily ingestion of nitrate and nitrite for the average American, and calculated the relative contribution of the various dietary sources. Vegetables were reported to be the major source, contributing

about 86% of the total daily nitrate intake of 99.8 mg. The rest were from cured meats, bread, fruits and juices, water, and milk and milk products (in decreasing order of percentage contribution to total nitrate intake). A total of 11.22 mg nitrite was estimated to be ingested daily. This was said to be made up of about 77% from saliva and 21% from cured meats.

3.5.3 Amines in foods

It appears that not much is known about the occurrence of various nitrosatable amines in foods (Sen, 1980; Shank, 1981a). Considering that there are more than 120 known carcinogenic N-nitroso compounds, Shank (1981a) felt that it is surprising that little is known about where and how often the precursors for these compounds occur in the human food supply. Some recent studies in this area have been reported by Ruiter (1973), Singer and Lijinsky (1976), and Neurath *et al* (1977) for a wide variety of foodstuffs. Besides dimethylamine and diethylamine, the most prevalent secondary amines appear to be pyrrolidine, piperidine, N-methyl-benzylamine, N-methylaniline and N-methylphenylamine (Neurath *et al*, 1977).

Fish appears to have been the most extensively studied for its amine contents. Fairly high levels of dimethylamine, trimethylamine and trimethylamine oxide have been reported for various fishes, particularly those of marine origin (Sen, 1980). The occurrence of amines in other foods, such as meat products, cheese and spices, have been reviewed by Sen (1980). The occurrence of amines in various spice premixes reported by Gough and Goodhead (1985) should be of interest since spices are commonly used in the preparation of various foods by communities in the region.

The possible occurrence of nitrosatable amines in a wide variety of other substances ingested, though strictly speaking not foods, should also be mentioned. A large number of drugs and medicines (e.g. the common medicines for colds that are antihistamines; oxytetracycline and aminopyrine), and many agricultural chemicals (such as many pesticides and insecticides) are nitrosatable amines or nitrosatable amino compounds. Many of them have been shown to give rise to the expected N-nitroso derivative on reaction with nitrite in acidic solutions (Lijinsky *et al*, 1972; Mirvish, 1975; Lijinsky and Taylor, 1977; Fine *et al*, 1977). It is thought that such reactions could be expected to take place to some extent when the amines are ingested by man (Lijinsky and Taylor, 1977).

3.5.4 Nitrates and nitrites in some Malaysian foods

Studies of nitrates and nitrites in local foods were carried out only in the 80's. Such data are extremely scarce. Berry, Augustin and Heng (1982) reported the study of these nitrosamine precursors in 10 commonly eaten vegetables.

Broccoli leaves were found to contain the highest amounts (4550 ppm) of nitrates, whereas cabbage had the lowest amount (146 ppm). Nitrates in the other vegetables were mustard leaves (*choy-sam*), 2875 ppm; mustard leaves (*kai-choy*), 2729 ppm; Chinese cabbage, 2331 ppm Amaranthus, 2146 ppm; *kang-kong*, 2031 ppm; white raddish, 1935 ppm; bitter gourd, 426 ppm; long egg-plant, 335 ppm. On the other hand, the nitrite content of these vegetables were found to be insignificant (< 1 ppm). It was however found that upon storage of mustard leaves, the nitrite content was found to have increased. The observations were said to suggest the importance of good agricultural practices and appropriate techniques of storage and processing to reduce the level of nitrate and nitrite in foods, particularly vegetables.

The nitrite content of 9 types pf pickled fruits and a pickled vegetables, *sawi cina (Brassica juncea)*, were reported by Abdul Salam Babji, Aishah and Aminah Abdullah (1984). Values were found to range from not detected in red cherry to 81.7 ppm, in red pickled pear. Seven types of salted fish studied had nitrite levels ranging from 11.7 ppm to 38.3 ppm. whilst *belacan* had a nitrite content of 12.7 ppm. Several types of meat products (sausages, burger, luncheon meat, etc.), local and imported, were also found to have nitrite levels below 200 ppm, in the range of 14.0 to 53.0 ppm. Higher nitrite levels of 100 to 165 ppm were reported for the samples of beefburger studied by Suhaimi Salleh (1983). This investigator also examined the effect of nitrite on selected pathogenic organisms.

3.6 Toxicity Of Nitroso Compounds

3.6.1 Acute toxicity

The acute toxicity of the simplest nitrosamine, dimethylnitrosamine, was described in man. The first documented human poisoning by this nitrosamine appears to be a report by Freund in 1937, in which the poisoning of two chemists involved in the production of dimethylnitrosamine for use as an anti-corrosion agent was described in detail. Studies were then carried out on mice and dogs, and the immediate and remote toxicological effects of the compound described. The author had suggested that the use of dimethylnitrosamine should be considered an industrial hazard (Freund, 1937). Subsequently, Barnes and Magee (1954) reported observations of two cases of poisoning due to the same compound, dimethylnitrosamine, in a laboratory of an industrial firm. These led the investigators to study the effect of nitrosamine on several experimental animals. A single lethal dose of the compound given by mouth or by injection was reported to produce extensive liver necrosis in rats, mice, rabbits, guinea-pigs and dogs, and death occurred one to several days after administration of the nitrosamine. It is these findings of Barnes and Magee (1954) that has led to widespread attention of investigators on the toxicity of the nitrosamine (Scanlan,

1975; Shank, 1981b). The acute pathological changes induced by nitroso compounds has been described by Magee and Swann (1969). The acute poisoning of other nitroso compounds, occurring mainly amongst chemists and other laboratory workers has been reviewed by Shank (1981b), illustrating what was once thought to be a problem limited to scientists and workers working closely with these compounds. It is now clear that nitrosamines (and their precursors) could occur widely in the environment and man's exposure to these poisons are now known to be extensive.

Little work has been done on the structure-activity relationship for the acute toxicity of N-nitroso compounds. However, it seems that acute toxicity decreases with chain length, of dialkylnitrosamines (Shank and Magee, 1981). The mode of action of the acute toxic effects of N-nitroso compounds has been studied by a few investigators. As reviewed by Shank and Magee (1981), it seems that the nitrosamines require metabolic activation to form the ultimate toxin. Furthermore, sites other than the liver has been reported to be affected, e.g. kidney, lungs, and testis. The nitrosamides, on the other hand, do not seem to require metabolic activation to an ultimately toxic form. In their review, Shank and Magee (1981) further pointed out that detailed studies on the acute toxicity of the nitroso compounds are lacking, probably because most investigators have focused on the striking carcinogenic properties of these compounds.

3.6.2 Carcinogenicity

After their study on the acute toxicity of nitroso compounds in experimental animals, Magee and Barnes (1956) went on to demonstrate the production of malignant primary hepatic tumours in rats fed with diets containing added dimethylnitrosamine. Since then, numerous studies on the carcinogenicity of nitroso compounds have been reported. It has been said that no class of chemical carcinogens has been studied as extensively in animal experiments as these compounds (Preussmann, 1972). Recent reviews on the subject include those by Magee and Barnes (1967), Preussmann (1973), Sen (1980), and Shank and Magee (1981). Some 130 N-nitroso compounds have been tested for carcinogenicity and at least 120 of them have been found to be strong carcinogens. Shank and Magee (1981) have tabulated all of these and indicated for each compound the target organs affected. In a separate table, these authors have tabulated the tissues affected and the various nitroso compounds known to affect them. They are said to be capable of producing tumours in virtually every vital tissues, although no one compound can induce cancer in all the tissues (Shank and Magee, 1981). These authors further pointed out that no other chemical class are known to have so broad a scope of tissues for carcinogenic attack.

It is known that nitroso compounds act as carcinogens in many animal species, including subhuman species; no animal species has been found to be

resistant to the carcinogenic effect (Preussmann, 1973). For example, the carcinogenic activity of dimethylnitrosamine has been studied and found to be potent carcinogen in at least 11 animal species (IARC, 1978a), diethylnitrosamine in 14 species, including subhuman primates (IARC, 1978a; Schmahl and Osswald, 1967), and methylnitrosourea (a nitrosamide) in 10 species (IARC, 1978a).

3.6.3 Possible human health hazards : epidemiological studies

Taking into consideration all evidences available, it is generally felt that man will probably react to nitroso compounds in a manner similar to that of experimental animals (Preussmann, 1973). This would mean that N-nitroso compounds are probably carcinogenic in man (Preussmann, 1973; Sen, 1980; Shank and Magee, 1981), echoing the earlier suggestion by Lijinsky and Epstein (1970) that these compounds "seem to be a major candidate class of carcinogens that are likely to be causally related to human cancer in industrialised society". There is really no debate as to the undesirability of these compounds (Scanlan, 1975). There has however been no direct evidence in the carcinogenicity on nitroso compounds in man (Preussmann, 1972; Sen, 1980). It may be difficult, if not impossible, to demonstrate in the general population a cause-effect relationship between exposure to the low levels of nitrosamines known to be present in some foods and the environment, and the incidence of certain human cancers (IARC, 1978b; Fraser *et al*, 1980; Sen, 1980). Nevertheless, several studies have indirectly examined the possible role of nitrosamine in the aetiology of a number of human cancers, some of which will be outlined below. The existence of correlation between environmental N-nitroso compounds and human cancers remains a working hypothesis (IARC, 1978b).

One of the earliest reports in this area was that of a possible association between oesophageal cancer in Africa and drinking locally distilled spirits thought to contain nitrosamines (McGlashan, Walters and McLean, 1968; McGlashan, 1969). The presence of the nitrosamines were however said to be unconfirmed (Shank, 1981b). Harington, Nunn and Irwig (1973) has reported detection of dimethylnitrosamine in the pooled cervical and vaginal discharge of South African women; the actual amounts present were not reported.

Since nitrates, which when ingested could produce nitroso compounds in the body, are known to be present in high concentrations in certain foodstuffs, it has been thought that populations ingesting large amounts of nitrate could be expected to have a high incidence of cancer of the relevant target organ (Fraser *et al*, 1980). Various studies have been directed to examining this hypothesis. Several studies in Chile, the country with the second highest age-adjusted mortality rates for stomach cancer in the world had examined and reported a correlation between gastric cancer mortality and per capita nitrogen fertilizer exposure in the country (Armijo and Coulson, 1975; Zaldivar and

Wetterstrand, 1975; Zaldivar, 1977).

Several studies in Colombia had examined the relationship between gastric cancer and nitrate in the drinking water. Preliminary studies by Correa, Cuello and Duque (1970) had revealed that residents in certain mountainous areas of south-west Colombia had a particularly high gastric cancer risk which correlated with the incidence of intestinal metaplasia, suggesting that the latter could be used as an indicator of gastric cancer risk. A series of investigations were carried out to investigate the possible environmental agents involved. In an attempt to determine the involvement of dietary nitrate in the development of gastric carcinoma Hawksworth *et al* (1975) observed that areas of high nitrate intake (as indicated by high urinary nitrate excretion) occurred where there were high levels of nitrate in the water supply, and in rural areas where the vegetables contain high nitrate levels. Further studies by Cuello *et al* (1976) and Haenszel *et al* (1976a) provided further evidences for the role of nitrate availability in the aetiology of stomach cancer. Tannenbaum *et al* (1977) had examined the evidences available and proposed two possible models of gastric cancer in Colombia, involving intragastric formation of carcinogenic N-nitroso compounds. In a later study, these investigators (Tannenbaum *et al,* 1979) reported, for the first time, high nitrite concentrations in gastric juice of individuals who have histologically proven gastric precancerous lesions, and who live in an area known to be at high risk to gastric cancer.

In a similar study in several towns in England, Hill, Hawksworth and Tattersall (1973) had observed that the town with the greatest consumption of nitrate (based on nitrate contents of foods and drinking water and monitoring urinary nitrate excretion) also had increased death rate from gastric cancer. The main route of exposure was thought to be the drinking water-supply to the town. A study in Japan had also examined the link between nitrate in water supplies and mortality from gastric cancer (Haenszel *et al,* 1976b).

The possible role of various foodstuffs in the development of gastric cancer have also been examined. Salted/dried fish, known to be rich in secondary amines, and pickled vegetables were said to be associated with the increased development of stomach cancer amongst Japanese migrants to Hawaii (Haenszel *et al,* 1972). However, the same investigators, were not able to confirm the observed association in a later study in Japan (Haenszel *et al,* 1976b). On the other hand, various other foodstuffs have been observed to be associated with a decreased gastric cancer risk, such as raw, green, leafy vegetables, and fresh fruits (Graham, Schotz and Martino, 1972; Haenszel *et al,* 1972; Haenszel *et al,* 1976b), which are rich in ascorbic acid, a known inhibitor of nitrosamine formation. The importance of lettuce in this respect has been particularly emphasized by Haenszel *et al* (1976b).

An extensive study by the IARC had attempted to determine the possible role of nitrosamines in the causation of oesophageal cancer (Joint Iran-International Agency for Research on Cancer Study Group, 1977). The study was

carried out in 15 specific areas of north-east Iran where this cancer was known to be unusually high. Major dietary items analysed for volatile nitrosamines, polycyclic hydrocarbons and aflatoxins did not show a significantly higher frequency of contamination than the nearby areas where the incidence of oesophageal cancer was not high. Similarly, nitrate and nitrite intakes did not differ between the high and low incidence areas. The investigators had pointed out that since several oesophageal carcinogens are non-volatile nitroso compounds, they would not have been detected by the methods used in the study. Bogovski (1976) and Fraser *et al* (1980) have suggested that although it has been shown that nitrate *per se* may not be incriminated as a cause of oesophageal cancer in Iran, the lack of nitrosation inhibitors in such an impoverished diet as was observed in the study areas could play an important role.

Another major study attempting to measure directly dietary consumption of nitrosamines and relate this to the development of oesophageal cancer has been conducted in China and reviewed by Shank (1981b). Although the findings were said to be inconclusive, Shank (1981b) feels that it is the only evidence available so far which associates to any degree environmental nitrosamines with a human cancer.

Thus, there has been no direct evidences of nitrates and nitroso compounds in the causation of human cancers. Fraser *et al* (1980) however feel that the epidemiological evidence available to date suggest that the hypothesis, that high nitrate ingestion is involved in the aetiology of gastric cancer, should not be lightly discarded. Shank (1981b) feels that the various studies now in progress should provide new information on the correlation between environment nitroso compounds and human cancers in the next few years.

3.6.4. Nitrosamines and nasopharyngeal carcinoma in Malaysia

Studies on the health hazards of nitrosamines to Malaysians are extremely scarce. The studies of Armstrong and co-workers (Armstrong and Chan, 1983; Armstrong *et al*, 1983) are amongst the few available on the subject. These investigators carried out a case-control study of nasopharyngeal carcinoma (NPC) among Malaysian Chinese to test salted fish consumption and other risk factors of the disease, including inhalants, the use of tobacco, alcohol, and nasal ointments. Interviews with 100 cases and 100 controls indicated that salted fish consumption during childhood was a significant risk factor; childhood daily consumption of this food item compared to nonconsumption carried a relative risk of 17.4. Occupational exposure to smokes (relative risk, 6.0) and to dusts (relative risk, 4.0) was also significantly associated with NPC. The two risk factors (consumption of salted fish and exposure to smoke and/or dust) were independent of each other. No association between NPC and tobacco, alcohol or nasal ointments was observed. The investigators suggested additional studies to identify potential carcinogens, such as N-nitrosamines, in salted

fish, where they may be introduced or accumulated in the various stages of fish processing, marketing, and meal preparation, and how they may be modified by chemical synergism with other foods in the diet.

3.7 Regulatory Measures

The purposes or the benefits to be derived in using nitrates and nitrites in foods, for example in a curing mix and as an antimicrobial agent has been discussed. The potential of nitrosamines as a carcinogen in human has been outlined. It is clear that the benefits that may be derived have to be weighed against the possible risks of these compounds as hazards to human health. The major environmental sources of nitroso compounds are indirectly the nitrate and nitrite and various secondary and tertiary amines in the human dietary and pharmacopoeia (Shank, 1981c). It would thus be quite impossible to totally eliminate them from our environment. Nevertheless, various measures may be taken to reduce such exposures, as well as the formation of nitroso compounds.

3.7.1 Control of nitrite in meat-curing

One of the measures that has been widely discussed is the control of the use of nitrite in meat curing. Lueck (1980) has pointed out that nitrite (usually sodium nitrite) is permitted in virtually all countries as additives for certain meat and fish products. In a number of countries, the maximum content of nitrite in food ready for consumption is not controlled by law. In the United States and United Kingdom, the content of residual sodium nitrite is limited to 200 ppm (200 mg/kg) (Lueck, 1980). The permitted amount of residual nitrite must be reduced to the minimum consistent with health and safety (Lijinsky, 1977). The use of nitrite in meat-curing in the United States shall be discussed below to illustrate the efforts taken by all concerned to bring about such control.

The use of nitrite was formally authorised by the United States Department of Agriculture in 1925, which stated that the finished product should not contain sodium nitrite in excess of 200 ppm (Binkerd and Kolari, 1975). According to these authors, who traced the study of nitrite in meat products from 1926 to 1974, initial monitoring of the levels of nitrite in various cured meats in 1926 revealed that the process in use at the time yielded extremely variable residual nitrite levels and, at times, high levels of nitrite in the product. Ten years later, in 1936, it was pointed out that much lower levels were reported, and most cured meats were well within the regulatory limits of 200 ppm nitrite. From 1970, there was considerable interest in the States and elsewhere in the amounts of nitrite in various cured-meat products, due mainly to the series of events outlined in the previous sections of this review. Various studies carried out between 1970 and 1974 showed that there was a marked

decline in the number of companies using nitrate in the various product categories. In addition, a marked decline in the highest amount of nitrate added to a given product by any one company was noted.

Havery, Fazio and Howard (1978) from the Department of Health, Education and Welfare, Washington, D.C., have been monitoring the levels of nitrite in various meat products since 1971. These investigators reported that the majority of meat products analysed were essentially free of 14 volatile N-nitrosamines within the limits of detection of the method used (10 ppb or 10 μg/kg). There was however one exception, and that is fried bacon. Efforts of various government and research laboratories, and the meat industry has had an impact on the processing of bacon products in that there has been a decline of nitrosopyrrolidine in the majority of commercial samples (Havery, Fazio and Howard, 1978). It had been claimed by the meat manufacturers that levels of 25 ppm of residual nitrite in bacon are now rather common (Lijinsky, 1977).

However, it has not been able to eliminate the contaminant, and some products still appear to have a problem in controlling the processing methods (Havery, Fazio and Howard, 1978). The estimate is that some 20–30% of the bacon produced is not able to meet this level of 25 ppm, and can contain up to 200 ppm residual nitrite (Lijinsky, 1977). Further studies and efforts into the use of nitrite in the processed meat industry are clearly necessary. In the meantime, new regulations on the use of nitrite and on the amount of residual nitrite allowable in meat seem to be needed urgently (Lijinsky, 1977). According to Shank (1981c), the United States Food and Drug Administration and Department of Agriculture are considering total or partial bans on the use of nitrate and nitrite as intentional additives to foods for human consumption.

3.7.2 Miscellaneous measures

Since nitrates in vegetables could be an important source of this precursor being ingested, this is an area where some control measures may be warranted. It has been suggested that the amount of such nitrate could be reduced if the amount of inorganic nitrate fertilizer applied to the soil is carefully controlled (Lijinsky, 1977). The author further suggested to reduce our exposure to nitrosable amino compounds, another group of potent precursors of nitrosamine formation, that may be present in food additives, drugs, pesticides and herbicides. It was felt that the safety of a drug which is a secondary or tertiary amine should be established only after it is fed together with nitrite in animal experiments so that an additional assurance of safety under normal intake conditions can be established.

Another line of approach that may be followed is to promote the wider use of inhibitors of nitrosamine formation, such as ascorbic acid (Lijinsky, 1977). Havery, Fazio and Howard (1978) felt that the decline in residual nitrite in fried bacon observed during 1971–1974 (outlined above) may be attributed to

improved control of processing, and reduction of nitrite used, as well as the increased use of ascorbate during processing. The incorporation of such inhibitors in other foods, including drugs, has been thought to be one of the possible means of reducing our exposure to nitroso compounds (Ziebarth and Scheunig, 1976; Sen. 1980).

Lijinsky (1977) had expressed optimism that the implementation of these measures might lead to the desired reduction in exposure. It is felt that there is hope that with a more cautious attitude, stricter evaluation and regulations, and more careful consideration of unknown biological consequences, we can reduce the incidence of cancer over the next several decades, as well as of other cumulative toxic effects.

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