Genotoxic Chemicals in the Human Environment: Their Identification and Interaction

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ABSTRACT

Epidemiology suggests that most cancers are induced by environmental factors. The use of screening tests which are dependent upon chemical interaction with DNA has greatly facilitated the identification of probable chemical carcinogens. Interpretation of the test results is never easy, in terms of human risk assessment, and is further complicated by the presence of other chemicals in an environmental context. Problems encountered are illustrated by examples of complex mixtures from three sources: air, water, and food. Difficulties in sample collection and preparation are addressed. Fractionation is desirable from an analytical viewpoint, but presents the danger that modifying factors may have been discarded in the non-mutagenic fractions. Thus, haemin or certain unsaturated fatty acids can reduce or abolish the mutagenic potential of various food components, at least in \textit{in vitro} tests. The relevance of such results to the \textit{in vivo} situation, however, has not been examined. Interactive mechanisms may operate at the pre-absorptive, absorptive, or pharmacokinetic phases of the mutagenic process, but the precise mechanism involved at any stage is seldom known. Mutagenicity research, as a branch of toxicology, is moving through two phases: (1) single compound testing, and (2) complex mixture testing, in which it is assumed that man breathes, drinks or eats mutagens as if these were fully independent processes. A third phase might be the synthesis of these independent processes for purposes of risk assessment. However, the usefulness of research that progresses further in this direction is questionable. Perhaps more imminently valuable would be the application of current knowledge to the reduction of general levels of mutagenic activity.

1 INTRODUCTION

Higginson (1976) argued from observations on migrant populations that it should be possible to reduce each site-specific cancer incidence to the lowest rate
found in a population. By summation of these lowest rates for different cancers, he estimated the theoretical minimum incidence of all cancers. By comparing this hypothetical minimum with rates observed in countries where cancer is common, Higginson suggested that 90% of cancers are due to environmental factors and are theoretically avoidable. Other estimates quote lower figures of around 50–60%. But can these cancers really be prevented?

According to Schottenfeld and Haas (1979), occupational cancer accounts for 1–5% of all human cancers. Therefore, a very substantial proportion of human cancers is environmentally induced and is not associated with the workplace. Control of carcinogens in the workplace involves a combination of engineering controls, personal protective devices to minimize exposure, substitution of less toxic materials, whenever possible, and enforcement of maximum allowable exposure levels. Such stringent controls are impracticable outside the workplace.

Almost half of the cancers in the British Isles occur in the lung, larynx, oesophagus, bladder, and pancreas (Doll et al., 1966). Evidence available suggests to at least some epidemiologists that smoking is responsible for most lung cancers and for a smaller proportion of the cancers at the other sites. Hence, by eliminating this one factor, the incidence of cancers could be greatly reduced (Doll, 1977; Doll and Peto, 1976).

The proportion of cancers associated with other known carcinogenic factors is small. This includes: the alcohol-related cancers (Adelstein and White, 1976), such as those of oesophagus and liver; occupational cancers, such as those caused by asbestos, vinyl chloride and β-naphthylamine; and iatrogenic cancers, such as those associated with cytostatic drugs, X-ray therapy and in utero exposure to diethylstilboestrol. Elimination of all these, however, probably would not reduce the total cancer incidence by more than 10% (Higginson, 1976). Any further reductions would be theoretically possible only following identification of environmental factors which may be responsible in part for other common cancers, e.g. cancers of stomach, colon and rectum. In the United States, about 35% of cancer deaths can be attributed to diet (Doll and Peto, 1981).

Many compounds have been identified as potential carcinogens because of their ability to induce DNA damage or increase the frequency of mutations in microorganisms, cultured cells, animals, and plants. The methods used have been validated to greater or lesser extents using chemicals of, apparently, known carcinogenic potential in rodents. Although these animal experiments have been criticized, 16 of 18 industrial chemicals or processes which are human carcinogens do induce tumours in rodents (Tomatis et al., 1978). Because of the low probability of a chemical reacting specifically with DNA in germ cells, inducing recessive lethal mutations and passing undetected as a health hazard in other toxicological tests, the following discussion will be based on the presumption that mutagenesis is important in toxicology only as a predictive tool for carcinogen identification. More than 13 years from the beginning of the expansion of work in mutagenesis, however, it is clear that the problem is far more difficult than the
mere identification of carcinogens, followed by legislation to remove them from the environment. What seems to be required to minimize cancer incidence is nothing less than cultural metamorphosis.

In most work, substantial doses of single chemicals are used, although no chemical is ever tested in isolation. The difference between testing a mixture and a ‘pure’ substance is one of degree and there is the possibility of interaction with components of the mixture, extractable substances from containers, particularly if they are made of plastic, and interference from the activation system. When very high doses are used, then the contribution of the inevitable impurities to the ultimate results grows in importance. An effect observed at a concentration of 1 mg/ml is not necessarily due to the quantitatively dominant compound under test. Some chemicals induce significant effects at concentrations of ng/ml. This type of problem may not be evident with pharmaceuticals, which are manufactured under strictly controlled conditions and where the major impurities are known and carefully monitored. The problem is greater with industrial chemicals which are not usually purified to the same extent; dyestuffs, for example, may contain as little as 50% of the named component. The possibility of interaction between compounds or their interference with the test increases again when environmental samples are tested. Samples from foods, air, water, or toxic waste dumps are chemically complex and the identification of their toxicological properties becomes fraught with difficulties. Simply separating mixtures and biologically analysing their components will not provide an accurate picture of how the active components would have reacted before they were separated.

Some problems encountered in carcinogen identification include the following:

1. The carcinogen(s) might not be genotoxic agents.
2. The carcinogenic potential may be due to a mutagen, but more than one might be present. The strongest mutagen in the test system used is not necessarily the most important from a human health point of view.
3. A potential human health effect may be the outcome of the damage resulting from exposure to several chemical and physical components in the mixture.
4. The mutagenic activity of a component in any complex mixture might pass unobserved because of competing, diverse, biological activities of other components. Alternatively, toxic components might reduce microbial test cell viability at concentrations of mixtures which are lower than those required to demonstrate mutagenesis.
5. A chosen activation system may be inadequate; for example, anaerobic metabolism is required in certain cases. Also, alcohol or aldehyde oxidations may be inadequate because the correct cofactor requirement has not been provided.

Products of Maillard reactions (which involve sugars and amino acids) contain mutagens and substances which increase gene conversion frequency in yeasts, but which are inactivated by S9 mix (Rosin et al., 1982; Wei et al., 1982). When heated
to normal cooking temperatures, arginine and glucose form mutagens, but the activity is low and it also is abolished by S9 mix (Aeschbacher et al., 1981). Maltol-ammonia, used as a model for these browning reactions, also forms a mutagen which is extractable with dichloromethane, but disappears when attempts are made to isolate the compound by thin-layer chromatography (Shibamoto et al., 1981). The convertogenic activity can be suppressed completely by Fe (III) and partially by Cu(II) (Rosin et al., 1982). Maillard reaction products can also interfere with the mutagenic activity of other chemicals, some of which require S9 mix for activation (e.g. aflatoxin B1), while others do not (e.g. MNNG, N-methyl-N'-nitro-N-nitrosoguanidine) (Stich and Rosin, 1982). Such interference with mutagenic effects has also been observed with haemoproteins which can bind benzo[α]pyrene, thereby reducing the concentration available for reaction with activating enzymic systems (Yagi and Akagawa, 1981). Chemicals of the same class can be responsible for modifying a mutagenic effect. Benzo[ε]pyrene is at most weakly mutagenic for Salmonella typhimurium TA 98 and TA 100, but it enhanced the mutagenicity of benzo[α]pyrene in strain TA 98 by up to 25 times when benzo[α]pyrene was 0.3 μg/plate and benzo[ε]pyrene was 1.5 μg/plate. On the other hand, there was no such enhancement in TA 100, where there was generally a tendency for antagonism (Hass et al., 1981). Theoretically, simultaneous exposure to a mixture of hazards may result in effects which are either interactive (synergistic or antagonistic) or non-interactive (independent and similar, joint action).

Furthermore, because of differences in the delivery of active metabolites to target cells in living animals, in vitro tests, even with mammalian cells, may not reflect accurately what can happen in vivo, where different rates of absorption, transport, metabolism and excretion may tend to fractionate the original mixture.

2 ATMOSPHERIC SAMPLING

2.1 Collection

Toxic substances can enter the body by three main routes: the respiratory tract, the skin, and the gastrointestinal tract. The first two are the more important in industrial environments.

There are two major groups of atmospheric chemicals: (1) vapour-phase chemicals, and (2) particulate chemicals. If the latter are below 10 μm aerodynamic diameter, then they are respirable and can be deposited in the lower alveoli. There is, however, a class of airborne particulates (< 0.1 μm) which behave almost as if they were vapour-phase chemicals. These are not deposited in the alveoli, but move in and out of the airways with respiratory movements. The procedures and instruments used in the study must be determined by the objective (for available equipment used in atmospheric sampling, see Air Sampling Instruments Committee, 1972).
It is strongly recommended that an industrial hygienist be consulted at the beginning of any study so that sampling conditions can be optimized, or at least standardized.

Factors to consider include the following:

(1) The weight of sample collected. This can be varied by the airflow rate through the sampler, the sampling time, and the type of instrument. An industrial process may have a fixed time of operation, in which case only the flow rate can be varied.

(2) The dust particle size. Total dust may be required, or perhaps only the respirable fraction, in which case a prolonged sampling time may be necessary. Patience, however, may be rewarded since there is an inverse correlation between mutagenic activity and particle size, in the respirable range (Figure 1) (Clark and Hobbs, 1980). Similar results have been obtained with airborne particulates in ambient air in an industrial town (Sorensen et al., 1982).

All sampling techniques lead to some fractionation of the atmospheric pollutants. This is inevitable and may be desirable, but a sample should be collected dry and stored dry, preferably under nitrogen in inert containers and in

Figure 1 The association of mutagenic potential with the aerodynamic diameters of six fractionated coal fly ash samples. From Clark and Hobbs (1980). Reproduced by permission of Alan R. Liss
the dark. These ideals are not always possible to meet, but, in that case, the influence of any modifying factors, such as solvent, should be investigated wherever possible. Dry sampling of airborne particulates in the respirable range is possible with an elutriator or a cyclone. Descriptions of these instruments are given in Air Sampling Instruments Committee (1972).

2.2 Sample Preparation

Having collected a sample, it is necessary to interfere with it in some way to bring it into a form which is acceptable to the animals or cells during a test. Any solvent used can modify the toxicity of the particulates. If the rapid elution of available mutagens is the objective, then dichloromethane or benzene–methanol are the solvents of choice, whereas a physiological fluid may be more representative of the in-life situation. Early studies (Chrisp et al., 1978) suggested that a physiological fluid, such as horse serum, may be a better solvent for the extraction of mutagens from power station fly ash than more conventional solvents.

This has not been adequately confirmed by other laboratories. King et al. (1981) compared the release of mutagens from diesel exhaust particulates in the presence of organic solvents, lung fluids, lung cytosol, and human serum. Of the physiological fluids, serum and lung cytosol were more effective than acellular lung lavage fluid (Figure 2). But the most efficient extraction of mutagens (for S. typhimurium TA 98) was achieved by dichloromethane.

Wei et al. (1982) compared three methods of mutagen extraction from fly ash:

1. horse serum for seven days at 37°C;
2. dimethylsulphoxide (DMSO) for 45 min at 37°C; and
3. azeotropic benzene–methanol mixture (60:40, w/w) with ultrasonication for 15 min in an ice bath, repeated three times.

The benzene–methanol mixture extracted most mutagenic activity, while serum generally extracted least (Table 1).

In another study on airborne particulate matter (Takeda et al., 1983), 40% more mutagenic activity was recovered by extraction with DMSO for 24 hours at 37°C than with foetal bovine serum, under the same conditions. These authors showed that at least five classes of serum proteins have the capacity of transporting 'direct-acting' mutagens. It was also suggested that neutral, polycyclic aromatic hydrocarbons (PAHs) are associated with β-lipoproteins, while the acidic fraction is associated with both α- and β-lipoproteins. The extraction efficiency of a particular batch of serum may depend, therefore, upon the physiological condition of the horse or bovine from which the serum is derived. Problems in comparing results from different studies are partly due to the differences in extraction methods used.
Table 1  Mutagenic potential of various types of extracts from fly ash samples tested in S. typhimurium

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<tr>
<th>Filtrate</th>
<th>S9 Control rev/plate, ( \bar{x}_g ) (GSE)</th>
<th>S9 Fly ash net rev/plate, ( \bar{x}_g ) (GSE)</th>
<th>S9 SA, net rev/( 10^8 )mg/cells</th>
<th>TA 98 Control rev/plate, ( \bar{x}_g ) (GSE)</th>
<th>TA 98 Fly ash net rev/plate, ( \bar{x}_g ) (GSE)</th>
<th>TA 98 SA, net rev/( 10^8 )mg/cells</th>
<th>TA 98 nr1 Control rev/plate, ( \bar{x}_g ) (GSE)</th>
<th>TA 98 nr1 Fly ash net rev/plate, ( \bar{x}_g ) (GSE)</th>
<th>TA 98 nr1 SA, net rev/( 10^8 )mg/cells</th>
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<td>37 (1.08) 75 (1.03) 4.8</td>
<td>27 (1.11) 74 (1.09) 5.9</td>
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<td>22 (1.12) 77 (1.05) 6.9</td>
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<td>36 (1.06) 59 (1.05) 2.9</td>
<td>25 (1.23) 123 (1.07) 12.3</td>
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<td>19 (1.04) 140 (1.03) 15.1</td>
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<td>36 (1.01) 119 (1.02) 10.4</td>
<td>25 (1.19) 138 (1.05) 14.1</td>
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nr denotes nitroreductase-deficient strains.

SA = specific activity = (number of fly ash revertants - number of control revertants)/8 mg (for 10^8 cells).

\( \bar{x}_g \) = geometric mean number of revertants/plate.

GSE = geometric standard error.

From Wei et al. (1982). Reproduced by permission of Alan R. Liss.
2.3 Mutagens

Airborne particulates are carriers of a wide variety of chemicals, including some mutagens and carcinogens. While PAHs may be important, the mutagenic activity of particulates is not always correlated with benzo[a]pyrene (Tokiwa et al., 1980). Of the fractionated mutagenic activity, about half was associated with the neutral fraction, but some acidic fractions showed very high activity in S. typhimurium TA 98 when in the presence of rat lung S9 mix. These same fractions exhibited lower activity in the presence of rat liver S9 mix. In an analysis of urban airborne particulates in California, Salamone et al. (1979) showed that many PAHs were present and that nine of these were mutagenic.

Dusts in iron foundry air carry at least 50 PAHs (Skytta et al., 1979), which are formed during processing from the organic additives in the moulding sand. While all samples were mutagenic, there was no correlation between benzo[a]pyrene concentration and the mutagenic response. This could be due to the presence of...
other substances with activity greater than benzo[a]pyrene, or the additive or potentiating effects of other components.

In certain types of airborne particulate, nitro-organics have been identified, either as a class (using nitroreductase-deficient variants in parallel with the standard S. typhimurium strains) or individually. Nitro-organic compounds also occur in coal fly ash (Wei et al., 1982).

Inorganic substances may be important in dusts. Hexavalent chromium is responsible for the mutagenic fumes produced by the metal in the inert gas welding of stainless steel (Stern et al., 1981). The activity may be lowered by the presence of lower oxidation state ions (e.g. Fe(0), Fe(II), Al(0)).

3 WATER SAMPLING

3.1 Collection

Collection of representative water samples poses problems at least as difficult as those involved in atmospheric sampling. Factory effluents entering a surface water system can vary greatly in their composition throughout the day and week. Processes within a factory may be operating at high output for some months only to be replaced by other processes. The manner in which effluents are treated may change. Chlorination is commonly used, but this may be supplemented with sulphite treatment or entirely replaced by ozonization. Finally, before released to a lake or river, the water may or may not be passed through charcoal to absorb many of the organic components. Once in the lake or river, there is ample opportunity for the effluent to interact with effluents from other sources, with naturally produced organic components, or with other factors such as sunlight, dissolved oxygen, suspended or sedimented particles.

The conclusion of one epidemiological study (Kuzma et al., 1977) was that there is an association between cancer and the presence of pollutants in treated surface water as opposed to treated well-water. This contention was supported by studies demonstrating a statistical association between the consumption of surface water and increased risk of gastrointestinal tract and urinary tract cancers (Harris et al., 1977). Laboratory studies have demonstrated that non-volatile mutagens appear in drinking water as a result of chlorination and that sulphite treatment reduces this activity (Cheh et al., 1979). Field studies have confirmed that treated drinking water has a greater mutagenic potential than raw, untreated water (Schwartz et al., 1979). Conventionally (activated sludge) treated waste waters may subsequently pass to advanced waste water treatment plants, which use a variety of physicochemical treatments (Saxena and Schwartz, 1979). However, water from such plants still shows mutagenic potential in the Ames test. Short periods (1–4 min) of ozonization can inactivate several mutagens (e.g. acriflavine, proflavine, β-naphthylamine, benzo[a]pyrene, 3-methylcholanthrene, 7,12-dimethylbenz[a]anthracene, aflatoxin B1, sodium azide, bis(2-chloroethyl)amine); certain other chemicals are unaffected by the ozone treatment
Methods for Assessing the Effects of Mixtures of Chemicals

(e.g. β-sultone, MNNG) (Burleson et al., 1979; Caulfield et al., 1979) and 1,2-dimethylhydrazine is activated to a bacterial mutagen by such treatment (Chambers and Burleson, 1982). The induction of sister chromatid exchange was demonstrated in organic concentrates of tap water from several American cities except when distilled or charcoal-filtered water samples were tested (Guerrero and Rounds, 1979).

3.2 Sample Preparation

Concentration of water is normally required and involves several steps:

(1) filtration to remove particulates (e.g. through filter paper). This step might be followed by:
(2) solvent extraction, reversed osmosis, flash evaporation or adsorption onto an ion-exchange resin (XAD-2 is commonly used);
(3) elution from the resin with a volatile solvent (acetone or methanol);
(4) removal of the solvent by evaporation at moderate temperatures, followed by redissolving the residue in a known volume of another solvent compatible with the biological test system (dimethylsulphoxide).

Various methods for concentration/extraction of surface waters and effluents have been compared (Dutka et al., 1981). Unfortunately, each of the steps is capable of modifying the mutagenic potential of the native water, either by fractionation or by interaction with the mutagens. For example, extracts which are dried may have greater mutagenic activity than extracts which are left in solution (Williams et al., 1982). There are two possible ways in which these difficulties can be minimized: by using either unmodified water or standardized methods of preparation of the water for testing. Freeze-drying and XAD-2 adsorption techniques provide different kinds of samples. There is much more organic material recoverable per unit volume of water using the freeze-drying technique, but its use is not favoured because it is so slow and, with normally available apparatus, only relatively small volumes of water can be processed.

3.3 Mutagens

Chlorination of water supplies contaminated with organic pollutants leads to the generation of potentially carcinogenic chlorinated hydrocarbons (Kraybill, 1978). Many of these compounds are also mutagens (Simmon et al., 1977) when tested in isolation. Of 71 chemicals tested, 45 were not mutagenic, while 26 were mutagenic in one or more assay system (either gene mutation in S. typhimurium or gene conversion in the yeast S. cerevisiae). Chlorinated organic compounds were prominent in this list of mutagens, but mutagens may be present in partially purified water before chlorination (Grabow et al., 1981). Also, the mutagenic resin
acid, neoabeitic acid, has been demonstrated in effluents from wood pulp mills (Nestmann et al., 1979).

4 FOOD SAMPLING

4.1 The Magnitude of the Problem

Man can absorb significant quantities of carcinogens and mutagens as a consequence of eating. The average daily food intake in the westernized, well-fed world is 1500 g wet weight and the average liquid intake is 1 litre (Sugimura et al., 1977). This material contains carcinogens/mutagens which are:

(1) of primary food origin (e.g. flavonoids such as quercetin and kaempferol);
(2) by-products of desired fermentations (e.g. nitrosamines in beer);
(3) products of microbial activity resulting from poor storage conditions (e.g. aflatoxin);
(4) introduced as non-nutritional constituents of marketed foods (e.g. certain dyes);
(5) introduced during terminal food processing prior to consumption (e.g. Maillard reaction products, polycyclic hydrocarbons and their derivatives, amino acid pyrolysis products); or
(6) formed during consumption (e.g. nitrosamines in the stomach, from secondary amines and nitrite at low pH).

These substances include the more potent experimental carcinogens/mutagens and some of them have been implicated in human carcinogenesis (e.g. aflatoxin B1).

Some interesting comparisons have been made in Japan of the diet in two areas: Akita, with high gastric cancer mortality, and Iwate, where the gastric cancer mortality is low. The most common procedure has been to make up 'typical' diets for each region, homogenize these and test water- and acetone-soluble extracts either for mutagenic activity in S. typhimurium TA 98 and TA 100 (Kamiyama, 1979; Michioka et al., 1979) or for DNA damaging potential in the Bacillus subtilis rec assay (Sasaki et al., 1979).

The rec assay indicated active substances were present in the diet only in the acetone-soluble fraction. Positive responses were higher in the Akita samples, in both the presence and absence of S9 mix. Michioka et al. (1979) found more than half of the Akita diet acetone samples to be mutagenic, while only 14% of those from Iwate showed activity. Similarly, Kamiyama (1979) found 13/26 Akita samples were mutagenic while only 5/36 Iwate samples were active. The mutagenic samples contained high proportions of roasted meat and fish, salted fish gut, beans and pickled or fried vegetables, while the non-mutagenic samples contained high proportions of bean curds, milk and raw vegetables. While nitrite
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congenital concentrations were high in the mutagenic samples, the non-mutagenic samples were high in nitrate, vitamin A and ascorbic acid.

Mutagenic activity has also been demonstrated, for example, with broiled fish, soy bean sauce, coffee and green and black tea. The activity in coffee is enhanced by manganese ions and destroyed by S9 mix (Friederich et al., 1982).

Besides the type of food, the manner in which food is prepared can have a significant effect upon the carcinogen/mutagen composition of a diet. Beef that has been fried, broiled or boiled contains mutagens specific for S. typhimurium TA 98 in the presence of S9 mix (Spingarn and Weisburger, 1979). During cooking there may be a lag phase when no mutagens are formed. This phase may be 5 min when frying, 35 min when broiling and 48 hours when boiling to prepare beef stock. Temperature and time of cooking are important factors in the formation of these mutagens, as demonstrated with pan-fried hamburgers (Pariza et al., 1979).

Much of the mutagenic activity in cooked foods seems to be due to certain amino acid pyrolysis products, particularly those of tryptophan. Also, powerful, mutagenic quinoline derivatives have been isolated from broiled, sun-dried sardines (Nagao et al., 1981). Antimutagens also are present in certain raw vegetables, but cooking may destroy them. Non-enzymic browning reaction products may possess antimutagenic potential, e.g. the mutagenic activities of MNNG and aflatoxin B₁ were reduced by autoclaved lysine–fructose mixtures and caramelized D-sucrose (Chan et al., 1982). Inhibition of mutagenesis has also been demonstrated with haemin (Hayatsu, 1982), oleic acid, and linoleic acid (Hayatsu et al., 1981). The action of haemin is both on the microsomal activation system and directly upon the mutagens. A precipitable complex is formed between haemin and the tryptophan pyrolysis product, Trp-P-2. However, activity is not inhibited with all mutagens (Table 2). Since haemin is insoluble in ether, it is quite likely that normally it will be separated from many of the food mutagens during the usual extraction procedures. While this separation may be considered to imbue the in vitro tests with artifactually high sensitivity, a similar separation is likely to occur in the gastrointestinal tract during food digestion and absorption. Consequently, the antimutagenic activity of the unsaturated fatty acids may be of more importance. Oleic and linoleic acids (but not stearic and palmitic acids) can inhibit the mutagenicity of various mutagens, including various amino acid pyrolysis products, aflatoxin B₁ and benzo[a]pyrene. It is possible that these fatty acids are responsible for the difficulties which exist in demonstrating any mutagenic potential in the Ames test with organic extracts of aflatoxin B₁ in contaminated ground-nuts (Dr J. Hope, Unilever Research, personal communication).

Other, even greater, complexities are possible. Several naturally occurring phenolic compounds, such as chlorogenic and gallic acids, are potent clastogens and inducers of recombination, but they can also strongly inhibit the endogenous formation of N-nitroso compounds (Stich and Rosin, 1982). These examples serve to illustrate the importance of examining the mutagenic potential of a diet.
Table 2  The interaction of haemin with mutagens in the Ames test

<table>
<thead>
<tr>
<th>Mutagens inhibited by haemin</th>
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<tbody>
<tr>
<td>Trp-P-1</td>
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<tr>
<td>Trp-P-2</td>
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<tr>
<td>Glu-P-1</td>
</tr>
<tr>
<td>Glu-P-2</td>
</tr>
<tr>
<td>Amino-α-carboline</td>
</tr>
<tr>
<td>Aminomethyl-α-carboline</td>
</tr>
<tr>
<td>IQ</td>
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<tr>
<td>MelIQ</td>
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<tr>
<td>MelIQ&lt;sub&gt;x&lt;/sub&gt;</td>
</tr>
<tr>
<td>Activated Trp-P-1</td>
</tr>
<tr>
<td>Activated Trp-P-2</td>
</tr>
<tr>
<td>Activated Glu-P-1</td>
</tr>
<tr>
<td>Benzo[α]pyrene</td>
</tr>
<tr>
<td>Benzo[α]pyrene-4,5-epoxide</td>
</tr>
<tr>
<td>3-Methylcholanthrene</td>
</tr>
<tr>
<td>7,10-Dimethylbenz[α]anthracene</td>
</tr>
<tr>
<td>Crystene</td>
</tr>
<tr>
<td>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>2-Acetylaminofluorene</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
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<tr>
<td>Mutagens not inhibited by haemin</td>
</tr>
<tr>
<td>AF-2</td>
</tr>
<tr>
<td>MNNG</td>
</tr>
<tr>
<td>4-Nitroquinoline-1-oxide</td>
</tr>
<tr>
<td>Nitromin</td>
</tr>
<tr>
<td>N'-Methyl-N'-nitrosourea</td>
</tr>
<tr>
<td>N'-Nitroso-di-n-butylamine</td>
</tr>
<tr>
<td>Quinoxaline-1,4-dioxide</td>
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<td>Carbadox</td>
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From Hayatsu (1982). Reproduced by permission of University of Tokyo Press.

or a meal in its entirety rather than isolating and testing particular components only.

4.2 Sample Preparation

In some cases, whole diets have been prepared and homogenized, but more usually a single food source is homogenized, then extracted. Smoke from charring meat or fish may be collected, or charred portions of cooked food scraped off for processing. Homogenized food has been incorporated in nitrosation reaction mixtures before mutagenicity testing. Variation between samples of food is
certain to occur, so generalizations should be drawn only after examination of many samples. If cooking is involved, this must be very carefully monitored and the conditions recorded. The advice of a food technologist might be valuable at this stage. Also, the manner in which the food is eaten could be important. Perhaps it matters whether certain components are eaten simultaneously or not. A few hours’ lag between the ingestion of some components could make the difference between interaction and no interaction.

Extraction can be with a series of aqueous and organic solvents either with or without adjustment of the pH. Normally, these multiple extracts are tested separately for activity. This type of investigation can give useful information, but it is important that the results obtained be interpreted with care: pooling multiple extracts might give rather different results.

4.3 Mutagens

There are relatively large numbers of genotoxic agents in food. The daily intake of mutagens by individuals is approximately 1–2 g, an enormous quantity in view of the lack of association between food items and carcinogenesis (Stich et al., 1982), and this represents a dose of approximately 20 mg/kg/day. If these substances have any impact upon human cancer incidence, then it appears that negative interactions predominate, resulting in an overall antagonistic effect. These calculations oversimplify the situation, since carcinogenic potency is not taken into account, but they do hint at the importance of assessing the overall effect of a mixture, rather than only its components.

5 MECHANISMS OF INTERACTION

When studying the mechanism of an interaction resulting in modulation of mutation or DNA repair synthesis, it is important to be aware that these observed biological events may not be the ones which are modulated by the interaction. The stage(s) at which modulation occurs must be isolated in order that the interaction mechanism be identified. It may occur in the pre-absorptive, pharmacodynamic, or pharmacokinetic phases of the toxic process.

(1) Pre-absorptive phase. New chemical species may result from the interaction of chemicals or chemical and physical components brought together in an environment facilitating their reaction. Examples are the formation of (a) N-nitrosamines from nitrite and secondary amines at low pH, in the mammalian stomach (Sander et al., 1968), and (b) mutagenic photoreaction products in benzo[a]pyrene solutions exposed to natural light (Gibson et al., 1978). Similarly, benzil is mutagenic in bacteria if the tests are performed under fluorescent light, but not if the laboratory has amber illumination (McGregor, 1979).
Pharmacokinetic phase. The formation of reactive electrophiles or free radicals is an important process in genetic toxicology. Some of these processes, particularly mixed function oxidase reactions, have received detailed study, but others, which may be equally important processes where modulating reactions can occur, have received less attention.

(a) Microsomal enzymes are inducible by many chemicals (e.g. PAH, polychlorinated biphenyls, 5,6-benzoflavone, phenobarbital, etc.) which, therefore, are responsible for modulating the effects of many mutagens.

(b) Dimethylsulphoxide, a commonly used solvent in genetic toxicology, interferes with the activation of dimethylnitrosamine, probably by presenting a large quantity of alternative substrate to the demethylating enzymes (Sugimura et al., 1976).

(c) High oxygen tension medium diminishes the reductive potential of microsomal preparations in vitro, thereby inhibiting benzidine or o-tolidine release from bis-azo dyes (Robertson et al., 1982).

(d) Certain metallic ions decrease in vitro DNA synthesis rates and/or the fidelity of DNA synthesis or transcription (Niyogi et al., 1981). Their action may be to produce conformational changes at either the active centres on the enzymes or the reactive sites on DNA.

(e) Caffeine actions and the interactions in mutagenesis have been particularly well studied. The incidence of chromosomal aberrations can be enhanced by caffeine, both in vitro and in vivo. Thus, the yield of aberrant cells is increased by caffeine when cultured Chinese hamster cells are treated with mitomycin C, TEPA, thio-TEPA, maleic anhydride, or 4-nitroquinolone-N-oxide. In V79 cells, cisplatin-induced aberrations are greatly enhanced by non-toxic concentrations of caffeine. Cyclophosphamide-induced chromosomal aberrations in the bone marrow of Chinese hamsters are increased by caffeine, while ultraviolet radiation and caffeine act synergistically to induce non-disjunction and crossing over in Drosophila. The caffeine-sensitive enzymes have been identified as the post-replication repair type. Single-strand DNA break rejoining is inhibited following γ-radiation.

However, many interactive processes occur which have not been identified. Norharman, for example, potentiates the response of bacteria to many mutagens and it appears that the effect is in some activation step (Fujino et al., 1978). Haemin and unsaturated fatty acids decrease the mutagenicity of amino acid pyrolysis products, but the mechanism by which this is achieved is unknown. Metabolic steps may be involved, but haemin also seems to interfere with the reactivity of benzo[a]pyrene-4,5-oxide with bacterial DNA.

A problem which is of great significance is the importance of these in vitro mechanisms to the in vivo situation. The pre-absorptive phase in particular is more complex in vivo than in vitro, there being a much greater chance in vivo for
the separation of components of a mixture. There may be differences in absorption (from the gastrointestinal tract, skin or lung), transport mechanisms, distribution, metabolic rates, and elimination rates and routes. These are factors which make the in vivo examination of mixture interactions extremely important before the scaling of risks associated with particular mixtures is made known.

6 CONCLUSIONS

In the earlier years of mutagenicity research, work concentrated upon essentially single compound testing. Interactions were of little real concern in this primary phase, when mutagen identification was the main objective. Now, although this primary phase has not been abandoned, more studies are being directed towards the effects of complex mixtures, as the secondary phase develops. It is being assumed in this secondary phase that man breathes, drinks, or eats mutagens as if these were fully independent processes. Clearly, this is not true, but the tertiary phase, in which investigations are directed towards the interaction of various complex mixtures to produce a resultant mutagenic potential which is a hazard for man, must be set aside for the time being.

Indeed, it is legitimate to ask, how far should even this secondary phase in carcinogen/mutagen evaluation be taken? The principal medical value of current mutagenicity research is the identification of potential carcinogens, while the subject has little to say at present about either the potency or possible interactions with other chemicals. Many of the published investigations of complex mixtures have tended to be analytical and concentrate upon the contributions made by known mutagens to the final level of activity. That is one useful approach. Another is to apply the knowledge already in our possession to the reduction of the general level of mutagenic activity to which we are exposed.

It is known, for example, that treatment of water supplies is both necessary (in order to protect against life-threatening microbial disease) and instrumental in generating mutagens in polluted surface waters (when chlorination is the treatment process). This information is sufficient to tell us that water pollution should be reduced and that alternative methods of water treatment should be more fully investigated. Neither course of action is easy and each is being followed.

What is not required when dealing with this problem is unbalanced concern about the presence of particular polycyclic hydrocarbons or particular alkyl/aryl halides. Similarly, air sampling researchers suggest that overall emission control, particularly of respirable particles, is required rather than attempts to reduce concentrations of certain chemical species.

When we extend our overview to food mutagens, the solution to reducing their level is again obvious, although there is much understandable reluctance to taking the required action. Food sources, cooking methods, and dishes are extremely important facets of cultural identification so in our efforts to safeguard the health
of society we should not be blind to the need of an individual to identify with his own group. One of the pleasures of a multinational and multiracial society is the opportunity it affords for sampling one another's cultures. While it is our duty to educate people in ways of reducing the carcinogen/mutagen level in our foods, this should be done with delicacy and tact. We should guard against toxicology being used, intentionally or not, as an instrument of social unification, so the danger then exists in it being discredited merely as such an instrument, particularly if what is offered is an alien cuisine which is bland, dull, and rootless.

Quite clearly, the problems of control of complex mixtures are considerably more difficult than those of the supposed single compounds which have been tested. Furthermore, the significance of a mutagenic complex mixture is more difficult to define. Because, in mutagenesis, submammalian and in vitro test systems are commonly used, extrapolation of results to man takes on a further complication over and above the problems already recognized elsewhere in toxicology. The parallelogram approach offered by Sobels (1977, 1980) is a worthwhile attempt to overcome this additional hurdle, but careful mutation work and the availability of radiolabelled compounds which allow estimation of dose to target molecules are requirements for completion of the parallelogram. To attempt the application of these techniques with complex mixtures is a daunting prospect, but perhaps it is the one which is required most of all; it may be in these complex mixtures that we shall find the environmental components involved in the origins of human cancers.

7 REFERENCES

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