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SYMPOSIUM ON 'NUTRITION AND TOXICOLOGY'

Evaluation of the safety of foods

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When any doubt has been expressed about the safety of food it usually centres around the use of many non-nutritive additives and food itself has seldom been investigated. At the time when attention was first drawn to additives this was possibly quite understandable as food was very traditional and variety was just coming into a diet restricted during World War 2. After the early work of the USA Food and Drugs Administration (Lehman *et al.* 1949, 1955), scientists in Europe really began to question the use of food additives in 1953, possibly because of the report on chemicals in food and cosmetics which followed the Delaney hearings in the USA (Delaney, 1952). The resulting United States legislation has had world-wide influence and is still the subject of hot debate.

In considering today's talk I am reminded of the situation as I saw it more than 10 years ago when I discussed food additive testing and wondered what would have been the situation if an equal interest had been stimulated in the part played by food and its natural constituents on the health of man (Philp, 1968). Would we have a better perspective on the relevance of high dose toxic effects to the presence in food of small amounts of the chemical causing the effect? Would we have given greater prominence to public education to prevent food poisoning of bacterial origin? However, this is still a matter for speculation and the facts are that additive assessment has held the scene and it has unfortunately not progressed very far except that more tests are being requested.

Additives of all classes are used essentially for the benefit they confer on a foodstuff, to maintain the nutritive value, to improve the appearance or organoleptic qualities of the product and to present it to the consumer in a better form, either at the point of sale or at time of cooking or eating. A wider distribution of certain classes of food, availability out of season or greater convenience in cooking all can accrue from the use of additives.

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When additives were first tested it was the opinion of the American FDA that a food additive should be a harmless substance, one incapable of damaging test animals even under most stringent conditions. However, in 1960 the United States' Congress passed amendments to the Food and Drugs Act which established the safety-in-use principle. Additives no longer had to be harmless at any dose; they had to be safe under the conditions of use for which they were approved. The one exception to this was that no chemical that caused cancer in appropriate tests could be used in any quantity in food. Now although the second, cancer clause, has achieved great notoriety, it is even more important to understand the significance of the 'safe under the conditions of use' requirement.

Philosophy of testing

The conditions-of-use requirement is a most important point as it needs careful consideration in terms of how to study proposed additives. Should one only test under conditions close to use in order to meet this legislative requirement, or should one deal with the subject in two stages? First, to find if there is toxicity, then to find out how relevant any toxicity is to the intended use and provide evidence for safety-in-use or freedom from hazard. This would be despite the demonstration of toxic potential at higher dose levels. I believe that nowadays there is little doubt that it is this two-stage process which is required but there is a very important proviso and that is, that the attempt to determine toxicity is not carried to extremes. This is particularly important in today's climate of increased open forum type of debate. However desirable in principle this test philosophy may be, it can give rise to problems both for some expert committees and also, more particularly, for lay scientists, the public and the media.

However, if an adverse effect is demonstrated it can be examined in detail and there can be greater certainty of its absence at lower levels of exposure. This greater certainty is of value in extrapolating to man and deciding that its eventual use by the public is free from hazard. This does not mean of course that one should give unphysiological levels to animals after early tests have demonstrated that a non-nutritive chemical has little biological activity. One can of course give unphysiological levels of a food and this also has to be avoided.

The chemicals being tested

The chemicals being examined are usually quite different from other classes of chemicals normally subjected to toxicity testing. For example, a pharmaceutical is selected for a specific biological activity, tested first to show its effects against disease and then, secondly, for undesirable side effects. It is used in man at a level of biological effectiveness with a particular margin of safety. The risk of toxicity will be set against its effectiveness and the importance of the disease. Similar criteria relate to the use of pesticides which also require to have a biological action before they will be considered for use; they are distributed directly to the natural environment and conceivably could be present in the food of man as a contaminant.

In sharp contrast, food additives, most of which are non-nutritive chemicals, are chosen in the first place for their technological advantage, and only when proven in this field are their biological side-effects investigated. Biological activity and toxicity may be present but it is almost certain that they will not be present to the same extent as is essential in the case of pharmaceuticals and pesticides.

However, the problems posed are considerable because our technically desirable chemicals must have a very low level of biological activity in the form in which they are presented to the public. Their use will be unsupervised and the populations concerned can be extremely large. For this reason, early selection takes place so that chemicals are only accepted for food additive testing if they are of relatively low toxicity as shown by early acute tests. Usually only chemicals with a toxicity less than 1 g/kg body-weight would be accepted for testing.

Assessment programme

The most important question being asked in the very extensive programme of testing is whether or not it is reasonable to allow the chemical to be present even at low levels in the food of man where it may be consumed in some instances over the lifetime of an individual. All aspects of the programme have to be directed towards this important question and once the results have been obtained, interpretation also must be directed towards showing freedom from hazard-in-use.

When considering programmes for evaluation, much is said about the responsibility of the investigator to use judgement, but it is quite clear that over the years this simply has meant that any special tests can be added but none of the so-called guidelines can be ignored.

My example of a food additive programme gives the basic requirements and also indicates the use of special tests related to the nature of the additive and also some tests carried out to keep pace with academic thought and work which could influence future guide-lines (Table 1; Philp & Jenkins, 1961).

This work was done in the late 1950s and early 1960s to evaluate an emulsifier, glyceran polyricinoleic acid ester (Polyster WOL), which would be used in chocolate couverture and block chocolate. A programme of this scope is, of course, only possible with the combined efforts of graduates in several disciplines. Contributing to this work were biologists, biochemists, chemists and graduates in veterinary and human medicine. Most important are the analytical resources to ensure reproducibility of manufacture and maintenance of specification so that what is tested is similar to that which is sold. Research analytical work was most important at the stage of radio tracer application for the direct metabolic study. The principal headings of the programme give the general cover of the investigation. This serves to ask a number of questions, how toxic is the chemical, what organs are affected, is an effect reversible, is an effect toxic or adaptive, how is the chemical digested, absorbed, excreted and is there any storage or accumulation in the animal body? Will long-term use have an effect on the reproduction of the parents or of the young born to them? Is there any possibility of cancer as a result of eating for a lifetime or even from being in contact with the

Table 1. *Programme for biological evaluation of glyceran polyricinoleic acid ester (polyster WOL)*

Acute toxicity
Rats, mice, rabbits, chickens, guinea-pigs

Subacute toxicity
Rats
30 week feeding trials, 9% Polyster
45 week feeding trials, 9% Polyster
Liver function studies, haematology, pathology
*Lack of effect on serum cholesterol
*Lack of effect on red cell fragility
90 d tests, 0, 1, 2, 4 and 8% Polyster
Biochemistry, haematology, organ weights, pathology

Chicken (non-rodent) tests
90 d tests, 2, 5, 10 and 15% Polyster
Biochemistry, haematology, organ weights, pathology

Metabolism of Polyster WOL
Indirect:
*Absorption and metabolism by energy-restricted rats
*Carcass composition after feeding 9% Polyster
*Lack of effect on digestibility of groundnut oil or protein
*Lack of effect on lipase digestion of groundnut oil
*Lack of chylomicronaemia after feeding Polyster

Direct:
Distribution of [^{14}C]polylgycerol by whole-body autoradiography
Studies with ^{14}C-marker fatty acids ([1-^{14}C]oleic acid and [1-^{14}C]stearic acid) condensed with ricinoleic acid and incorporated into Polyster
Studies with tritiated Polyster prepared from tritiated polyricinoleic acid

Chronic toxicity
Growth in three generations
Breeding performance in three generations
Pathology

Carcinogenicity
Rats:
2 year feeding, 5% Polyster
2 year cutaneous application test
†2 year co-carcinogenicity test
†2 year subcutaneous injection test

Mice:
80 week feeding, 5% Polyster
80 week cutaneous application test
†80 week co-carcinogenicity test
†80 week subcutaneous injection test

Human studies
Digestion and absorption
Tolerance of high dose.

*Special non-standard tests.
†Tests of debatable value.

material during manufacture of food? Is the animal work at all relevant to the use in man and for this question appropriate metabolic studies require to be designed and carried out, preferably as early as possible in the study to avoid the use of an unsuitable test animal.

This entire programme is a good example of the study of a pharmacologically non-toxic additive and after all the work it was found in only one respect to differ from groundnut oil and that was in giving minimal liver enlargement. Subsequent tests gave a satisfactory no-effect level. The liver enlargement was not in any way pathological, no lesions due to the additive ever appeared in the liver during all the long-term and reproductive tests. Nuclear counts of liver cells and deoxyribonucleic acid estimations indicated that the change was one of cell hypertrophy. The liver reverted to normal after the additive was removed from the diet and it was considered that hypertrophy resulted from an adaptation to increased functional requirement. This was considered to be analogous to the enlargement seen during pregnancy (Wilson *et al.* 1970). Although it was not considered that this indicated harm to the animal or would be of significance in

man at the level intended for use, the no-effect dietary level was calculated from the amount giving no liver change in the most susceptible animal (2%).

Special test. One need not consider all aspects of the programme but one of the special tests may be of interest particularly to nutritionists. This was part of the indirect metabolism section and utilized energy-restricted rats. The test was developed to illustrate whether or not Polyster WOL was utilized as a source of energy by the rat. Previously it had been shown in the laboratory that the weights of 4-week-old rats remained fairly constant for 2 weeks if the diet was limited to 75 kJ (18 kcal)/d. The energy intake of rats given 9% Polyster WOL in the diet for 9 weeks was restricted for 17 d to a daily ration of 5 g fat-free diet providing 75 kJ (18 kcal)/d. When some of the animals were killed, the fat depots were found to be depleted, and carcass analysis for fat and free fatty acids confirmed that fat had been metabolized during the period of depletion. Subsequent feeding of Polyster WOL to the remaining animals gave complete recovery of weight in 14 d. We therefore provided evidence that the ester was utilized as a nutrient. However, when this work was reviewed by the UK authorities it was considered necessary to have more direct metabolic studies.

The subsequent metabolic studies utilizing radio isotopes showed that Polyster WOL was digested in the gut to give free polyglycerols, polyricinoleic acid and free ricinoleic acid. Lower polyglycerols (containing up to three glycerol units) were absorbed and excreted unchanged in the urine but higher polyglycerols were excreted in the faeces. Up to 90% of the fatty acid material was absorbed and metabolized. In this study one was really asking why the material was harmless.

Test of doubtful value. As seen from the Table, two tests were considered to be of doubtful value and I shall mention one. At the time when the programme began the subcutaneous injection of a test substance was considered to be an acceptable example of a stringent test and many scientists accepted this without question. However, despite the fact that benign or malignant cancer can certainly be produced in this way when a variety of chemicals are injected, there was growing belief that this was not a suitable test for food additives. Nevertheless, we carried out the test, despite the possibility that a false positive might have been produced, because it also gave us the opportunity to investigate the value of subcutaneous injection at varying sites as opposed to the then current use of repeated injections at the same site.

Fortunately, over the years, reasoned argument and experimental work (Grasso & Goldberg, 1966; Roe, 1966) has led to a change in the UK acceptance of the obligatory nature of subcutaneous testing. Now, if there is good evidence that an additive is absorbed in the test animal, then a feeding trial is the only essential test for carcinogenicity.

Additional tests for chemicals. This programme did look at some possible additional effects, for example, that on skin, and this was largely because the material could be expected to come in contact with the skin of workers in the food industry. However, we were under no obligation to include this in a 'food additive investigation'. I believe that even today this programme would be an acceptable

one but we would now have to take into consideration the information required before a chemical may enter industry irrespective of its end use.

A food additive programme is tied to the future use of the chemical but it has also to be appreciated that in the early 'formative' years of the chemical's life it will pass through a laboratory into a pilot plant of a factory and on to large-scale production. Therefore some attention would be required to be given to the properties relevant to exposure of workers.

For example, attention should be given to its potential for skin effects such as irritation and skin sensitization, even in some cases potential for systemic allergy if it is to be produced as a fine dusting powder which could be inhaled. A factory population, although not eating a food additive, could be grossly exposed to a dusting material. The recently developed series of tests for mutagenic effect are also for application at this early stage (*Lancet*, 1977).

Since the advent of European legislation and of the Health and Safety at Work Act in the UK, these aspects are all now included within the requirement for early information on a chemical's biological properties. In the past they seldom featured in what was referred to as a food additive testing programme. Those of us actually engaged in food additive examination and seeing some of the possible problems, have suggested ways of dealing with the question of allergy (Frazer, 1962; Frazer *et al.* 1962; Philp, 1968, 1974). Such suggestions could only be tentative at the time because well tried methods were then only in their infancy and experience in practical situations was lacking.

Now, however, highly predictive methods, at least for skin sensitization, are in regular use in industry for testing chemicals expected to contact skin and knowledge obtained will be valuable when considering possible ingestion (Magnusson & Kligman, 1970). At present it is accepted that if an individual has a skin sensitization to nickel, large challenge doses by mouth will exacerbate the skin condition. Even in this situation, however, there is a demonstrable safe level of exposure although extremely small. The humble lettuce can produce a skin allergy although no effect has been experienced when it is eaten (Krook, 1977).

Although methods for testing systemic allergy (type 1. Gell & Coombs, 1963) can still be used as an investigative tool, they are at the stage of posing a problem of interpretation and relevance. More attention is now being paid to the pattern of man's response to common food allergens as evidenced by the presence of circulating antibodies. As the various techniques in this field continue to be developed, it can be expected that more knowledge will be generated with greater relevance to the main constituents of food rather than to the additives.

Absorption and metabolism

I have indicated the importance attached to the absorption studies and also how important analytical developments can be. A useful example of just how important this aspect of a programme can be comes from studies on the artificial sweetener cyclamate.

Our laboratory became interested in this particular sweetener for purely commercial reasons and we were required to answer the question whether or not the material could fully replace sugar in the diet of man. For such an important question considerable attention had to be given to any suggestion that the information available at the time might be inadequate.

Cyclamate had been widely used and had been the subject of much evaluation work supporting its safety in use. However, largely because of improved technology, early work showing no absorption or metabolism in man and animals came to be questioned. Kosima & Ichibagase (1966) showed that in some rabbits, dogs and in one man, cyclamate was partially metabolized to cyclohexylamine (CHA). Leahy *et al.* (1967) showed that out of forty individuals who were tested, two excreted CHA in urine. This new metabolite had never previously been considered as a food additive, therefore the probability of its being produced during metabolism of such a widely distributed chemical was of some concern. However, there was also considerable confusion at first with regard to the interpretation of this work. There was even the suggestion that normal individuals excreted CHA in their urine in the absence of cyclamate in their diet. There was also some doubt as to the precise nature of the chemicals that might be present in any commercial sample of cyclamate, one suggestion being that if CHA were present initially this would have some effect on the metabolism of cyclamate in the body. There was also the suggestion that dicyclohexylamine, a suspect carcinogen, might also be present.

These questions immediately highlighted the need for extremely critical analytical tests to be developed so that it would be possible to ensure that not only was the specification for purchase of cyclamate adhered to, but that unequivocal evidence could be presented showing the presence or absence of metabolites of cyclamate in urine and faeces of animals and man. CHA was identified by comparison with a standard using gas–liquid chromatography and mass spectrometry, and the analytical method was first applied to screen the urine of 100 volunteers from the laboratory. This showed unequivocally that CHA was never present in the absence of cyclamate (Collings & Favell, 1971). With various dosing regimens it was shown that of 141 individuals examined, thirty-six excreted CHA after being dosed with cyclamate. There was considerable variation in what came to be known as the conversion rate, and it ranged from less than 1 to over 50% of the dose administered. Of some interest was the fact that with increasing doses of cyclamate the CHA produced did not increase in proportion although there was an increase in the absolute amount excreted. Further studies utilizing the antibiotic Ampicillin to control the gut flora, showed that the metabolism of cyclamate to CHA in man is probably due to a micro-organism in the gastro-intestinal tract. Similar studies in the pig and rat confirmed this site of conversion (Collings & Favell, 1971).

The demonstration of this interaction with the gut flora was not only of significance in interpreting the situation in man, but has to be considered in any retrospective consideration of animal information dealing with cyclamate. It also

would be required to be taken into account if further work were to be done on cyclamate in species such as the rat. It has been shown quite clearly that not all rats or stocks of rats have the same capacity for gut flora interaction although this can be transferred from one stock to another provided infection of the gut takes place. Any retrospective examination of cyclamate information has to take into account the question; was the animal given the chance to respond only to cyclamate or to cyclamate and cyclohexylamine? Quite naturally, this work has led to further examination of cyclohexylamine in its own right as a possible food additive associated with the use of cyclamate.

Food evaluation

Having referred briefly at the outset to the possibility that food might be examined, I return now to this consideration. Food derived naturally can be of quite variable composition depending upon the source of nutrients received during growth of the crop or of the food animal. Attempts to test food over a long period must take this into account. One of our earliest experiences of this problem was when we investigated irradiated ham over two years in rats and mice, and we believed we solved the problem by using a right leg of ham for one group and a left leg of ham from the same pig for the test group. We also had to give great thought to the mineral content of the diet because ham is deficient in several nutritionally important elements (Jenkins, 1961). Supplemented diets were therefore used because the object of the study was to examine the effect of irradiation on the wholesomeness of the food. It is therefore equally important to understand the question which one asks when one is investigating food as it is when one is investigating food additives.

There was of course another check on the possible variability in the food we were testing in this instance because we knew that the pigs themselves were considered to be free from disease and fit for human consumption having passed through the normal acceptance routine for human food. When therefore we come to consider food from novel sources, we must not forget the many checks and controls there are in our present food chain from behind the farm gate to our plates. Just as we now ask very critical questions on specification and purity of food additive chemicals, we should in the case of novel foods, ask how the food is grown and what the checks are for lack of disease or absence of contamination from any source (Philp, 1974). Food processing can of course be beneficial in removing possibly harmful materials as, for example, the reduction in trypsin inhibitor from vegetable protein (Orr & Adair, 1967) and the reduction of antigenicity of food by heating (Todd *et al.* 1957) and (Jenkins, 1962).

The possibility for the contamination of food from a variety of sources was demonstrated in our laboratory when we showed that groundnut meal contaminated with the fungus *aspergillus flavus* could contain the now well-known mycotoxin, aflatoxin (Lancaster *et al.* 1961; Philp, 1964). Growth in culture of this fungus would, under certain critical conditions, give rise to the aflatoxin but it was

possible to grow it with no toxin being present. The most significant aspect was that the toxin was effective as a carcinogen in the rats at 7 ppm and only required 6 months to produce cancer. It is perhaps of value in passing to emphasize that no programme of food additive testing could fail to pick up this quality of carcinogen which is now being considered to have an effect in man if the exposure is great enough (Doll, 1979).

Both food additives and foods which are proposed for future use and which come from novel sources should be examined to exclude this type of natural contaminant. This is preferable to waiting for the extremely difficult epidemiological study which is one other way of identifying that chemicals occurring in nature and present in man's food are not necessarily safer than those synthesized by man.

It is really impossible to discuss the testing of food additives or the assessment for freedom from hazard-in-use of a food without commenting on the need for carcinogenicity testing. However, as this is being dealt with as a subject in its own right during this symposium, I shall content myself with a brief comment only.

It is certainly true that one of the most emotive results which can come from a test of a food additive is one suggesting that it could cause cancer. But it is my belief that in considering whether or not a real problem to man exists, one should pay more attention to the sequence of events in the animal test which led to the eventual indication of cancer at post mortem. Tissue reactions which are reversible but which, under continued insult, may lead to cancer should not by themselves be regarded as indicative of a carcinogenic effect. Much of the trouble seems to stem from the use of extremely high level dosage and I will finish on this point.

Even in a test for cancer the dose level employed is extremely important. The test for carcinogenic activity should come as part of the programme of testing for all other aspects of toxicity. From the earlier tests there will most probably be evidence of toxic effect. From this information a dose should be chosen which has an adverse effect on test animals but which does not kill or so affect the animal as to shorten the duration of life. This dose will be many times greater than that to which man would eventually be exposed because the common safety factor used is one-hundredth of the dose which does not affect animals.

If the test substance has so little toxicity that only very large doses, say 5% of the diet, will affect animals then the cancer test should be done at levels related to the possible total consumption by man plus a safety factor. It is customary to use exaggerated assumptions for calculating the total consumption by man and in this way it can be expected that a wide margin of safety could be provided. Examination of the literature on cancer investigation indicates that there is little difficulty in identifying a truly potent carcinogen, these can be detected even at ppm in the diet in under 1 year, our own experience with aflatoxin was only one of several. It is important that it should be realized that additives so far tested most certainly do not possess the properties of potent carcinogens and it is equally important to appreciate that before a new food could be contemplated for use it

would be required to be appropriately tested for carcinogenicity potential, particularly if there was strong possibility of contamination. If it is possible to separate the contaminant or its source from the food and carry out appropriate tests for toxicity, then the programme could be considered similar to that for a food additive although the eventual outcome would be to evaluate the food.

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