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food - science and techniques

Reports of the Scientific Committee for Food

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REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD

ON

GUIDELINES FOR THE SAFETY ASSESSMENT OF FOOD ADDITIVES

(Opinion expressed 22 February 1980)

INTRODUCTION

Definition of food additive

Several definitions of a food additive exist in national and international legislation which differ according to the purpose for which they are intended. An example of a simplified definition is the one proposed by the Joint FAO/WHO Expert Committee on Nutrition in 1955 which states that "intentional food additives are non-nutritive substances which are added intentionally to food, generally in small quantities, to improve its appearance, flavour, texture or storage properties". A more recent and wider definition covering food additives, whether or not endowed with nutritive value, has been adopted by the Codex Alimentarius Commission and is quoted in the Fourth edition of the Procedural Manual issued in 1975. For the purposes of these guidelines any substance is regarded as a food additive which is added to food at any stage during manufacture with the purpose of changing its characteristics.

General principles governing the use of food additives

General principles for the use of food additives were elaborated at the first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) held in Rome in December 1956. These principles are listed in the First Report of the Joint FAO/WHO Expert Committee on Food Additives published in 1957. Similar general principles are applied by the Commission and by the EEC Member States in developing legislation on food additives.

Apart from these considerations other factors may influence any decision regarding the acceptability of a food additive. The existence of the comparatively infrequent phenomenon of human intolerance to substances foreign to the body cannot be ignored, whether this intolerance takes the clinical form of hypersensitivity or allergy. For certain classes of additives, e.g. flavourings, organoleptic considerations may be a primary consideration.

Before an additive is accepted for use in food, it should have been subjected to an adequate toxicological evaluation. Implicit in this evaluation is the assumption that the proposed level of use does not exceed the lowest level which is necessary to ensure that the desired results in food manufacturing practice are achieved. Additionally, there should be evidence of "need" i.e. that the proposed use of the additive would have technological advantages and confer benefit on the consumer, in other words it is necessary to establish the case for what is commonly referred to as need.

Food additives should be permitted by the appropriate authorities. Legal control should be accomplished through the enforcement of lists of permitted additives which effectively prevent the addition of any new additives to food, until an adequate basis for judgement of their freedom from health hazards has been established. Where necessary, quantitative restrictions can also be incorporated in the relevant legislation.

The use of an additive must not mislead the consumer as to the quality of the product or encourage faulty processing and handling techniques.

Considerations relating to "need"

A "need" for a food additive may be claimed if the use of the additive gives demonstrable technological, economic or other advantages of benefit to the consumer.

a) Technological need

In some food manufacturing processes the use of additives is indispensable and a technological need therefore exists *a priori*. For example, margarine cannot be manufactured without emulsifiers and jams cannot be made without additives controlling the pH.

b) Economic need

Additives such as preservatives and antioxidants enhance the keeping quality of foodstuffs thereby extending the shelf life, reducing wastage, and providing a wide range of foods for large urban populations at all times of the year at tolerable prices. The use of additives to enhance shelf life may be claimed to be the fulfilment of an economic need.

Other considerations

The aesthetic appeal of food is of importance and food, which is unattractive to the consumer will not be eaten and therefore wasted. Customs and traditions determine the expectations of the consumer and food which does not have the appearance and texture within the normal range of variation to which the consumer is accustomed, will be rejected. Organoleptic factors influence the acceptability of food by the consumer and are determining factors influencing the food trade. Although no absolute necessity can be established in every circumstance, it can be claimed that there is a psychological need for colours and flavours in some foods.

GENERAL PRINCIPLES FOR THE TOXICOLOGICAL EVALUATION OF FOOD ADDITIVES

Once the technological need and value to the consumer of a food additive has been established, a decision is required regarding the implications for the health of the consumer due to the presence of that additive in food. Even if additives have been consumed for many years by man without evidence of acute adverse effect, it is misleading to argue, that their use might well involve less risk to the consumer than new additives which, although tested on animals, have not previously been consumed by man.

As it is extremely difficult to establish whether continued consumption of an additive over many years causes chronic effects in man, its prior consumption by man cannot be regarded necessarily as additional evidence for safety.

In particular, it is necessary to determine by toxicological examination whether the substance, when used in the manner and in the quantities proposed, might be injurious to the health of those population groups whose pattern of food consumption or physiological status, e.g. age, pregnancy, makes them the most vulnerable ones. A number of procedures exist for conducting such toxicological examinations. No fixed programme should be laid down to be followed rigidly in every case, but a framework is proposed for planning a sequence of steps which enables the safety-in-use of a food additive to be evaluated. The toxicological examination provides the data for such an assessment. In general one must rely on experimental data derived from investigations in laboratory animals. If the biological action of a substance has been ascertained qualitatively and quantitatively in different laboratory animals, the likely effects on man can then be estimated by careful extrapolation. Where available, one may also use human data derived from medical use, occupational epidemiology or specific studies such as studies on critically exposed groups.

The procedures to be employed for the testing of intentional food additives and for interpreting the resulting data have been discussed in many national and international publications, since first enunciated by the Joint FAO/WHO Expert Committee on Food Additives in 1957. As the science of toxicology has advanced, so the proposed procedures have been modified and updated where necessary.

In any evaluation of food additives two stages are discernible. The first is concerned with the establishment of adequate and reliable qualitative and quantitative data describing the biological activities of the substance under examination. The second stage is concerned with the interpretation of these data, their correlation with other essential information on the physical and chemical properties, and with estimates of the exposure of the population. Since the consumer may ingest the substance daily over the whole of his life time, the maximum intake from all sources needs to be estimated, which will cause no obvious detrimental effect on the health of the individual.

General guidelines for the testing of food additives

It is only possible to formulate general guidelines defining the scope of the examinations required, their sequence and interpretation in terms of acceptable intake by man. Consultation with competent experts concerning the proposed examinations, the methodology to be applied, the method of reporting and the presentation of the evaluation is advisable.

In general the proposals in these guidelines are intended to apply to the evaluation of new, or the reevaluation of already established intentional food additives, directly incorporated into food and fulfilling a defined technological or other purpose (as already outlined on page 7). They may not necessarily apply in full to non-intentional additives, accidentally incorporated into food as a result of processing, packaging or carryover from raw commodities. These latter are considered under "Special Considerations" (page 21).

The diversity of chemical structure and technological function of additives precludes the establishment of a single uniform pattern of test procedures covering all substances. Each substance presents its own particular problems and the planning and conduct of the toxicological examination remains the responsibility of the scientist testing the substance. The examination must take account of all available knowledge regarding the substance and of any toxicologically related compounds.

The importance of physico-chemical information

A knowledge of the chemical structure, of the physico-chemical characteristics and of the spatial conformation of the molecule of a substance is important for the proper design of the appropriate toxicological test procedures. Thus knowledge of the volatility, solubility or pH of a substance will determine the nature of its administration to the experimental animals. Information about the chemical structure and, where relevant, of the spatial conformation will permit some conclusions regarding its likely biological activity. An example of a suitable scheme is given in Annex 7 of the Council Directive 79/831/EEC[1] even though that Directive is not confined to substances used as food additives.

The importance of specifications in safety evaluation

The material eventually subject to toxicological testing should correspond to the food additive to be used in practice by the food industry.

Toxicological tests, carried out on samples for which there are inadequate specifications, may later be found to be valueless for a proper evaluation of the materials to be used in practice by the food industry, while tests made on an unidentifiable material are worthless.

Thus a food additive must conform to appropriate specifications. This will ensure that it can be properly identified and that the presence of all, but particularly the harmful, impurities is reduced to acceptable levels. Consideration of the chemical structure, and the route by which it has been synthesized, will enable a search to be made for specific potential impurities whose presence might otherwise escape detection. If the method of manufacture of an additive is changed, any alteration in the nature and amount of impurities must be determined, and a revised specification produced if appropriate. In the elaboration of an additive specification consideration should be given to degradation products that may arise during formulation or storage, and to the possibility of interaction of the additive with other substances present in food.

Methods of analysis

In principle, an adequate method of analysis is necessary for identifying and determining the quantity of additive present in or on the food. If an analytical method is not available, it is necessary to consider, as an alternative method of control, factory inspection involving examination of weight records.

Laboratory diet and animal husbandry

Adequate information on these aspects requires a full description of the animal diet used in the biological studies. This should include the composition, the results of analysis for macro- and micro-nutrients, and of analysis for the presence or absence of toxicologically important contaminants, e.g. mycotoxins, organochlorine compounds, nitrosamines, pesticide residues, toxic heavy metals, etc. In addition a description is needed of the conditions of husbandry, distribution and environmental circumstances to enable an eventual evaluation of the experimental results.

Biological research

Information is needed on the absorption, distribution in the body, bio-transformation and excretion of the substance, and its metabolites, in laboratory animals, and, if possible, in man. Furthermore, the potential for causing toxic reactions needs to be fully explored.

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[1] Council Directive of 18 September 1979 amending for the sixth time Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, OJ L 259, 15.10.1979, p. 10

Testing normally starts with a determination of acute toxicity. In relatively rare instances the substances may exhibit unacceptable toxicity at this stage, leading to the abandonment of further studies. The information from these tests normally provides useful background and guidance for further tests.

Toxicological assessment should next proceed to an examination of genetic toxicity as an early screening measure to give warning of possible carcinogenic or mutagenic potential. Then the metabolism, including pharmacokinetics, of the substance should be examined. If the metabolic and pharmacikinetic studies reveal the formation of substances normally occurring in the body, a safety assessment may be possible at this stage. If any of the major metabolites are found to be substances with known toxic properties or with unknown biological activities, testing must continue for subchronic effects and chronic effects, where indicated. Studies on placental transfer, reproductive effects and teratogenicity are also needed. Any effects found at the stage of subchronic testing require evaluation to decide whether they will present a toxicological risk under actual exposure conditions.

The chronic toxicity and possible carcinogenicity of a substance always require further detailed study and evaluation, whenever the substance has a chemical structure suggesting the possibility of carcinogenicity, or shows effects in subchronic toxicity tests suggesting more serious consequences on longer exposure, or shows possible adverse effects in the genetic toxicity studies, or if the metabolic investigations suggest the production of reactive intermediate metabolites, or if the additive is likely to be consumed at a substantial level. The outcome of the chronic toxicity tests permits a final decision to be taken regarding the acceptability of rejection of the substance. For more detailed protocols of the studies considered below see Annex.

Any system being developed should attempt to organize the existing knowledge on safety assessment in such a way as to permit judgements at consecutive stages in the toxicological examination of a substance. As specified tests are completed, the toxicologist should decide whether a decision on safety can already be taken in the light of the results obtained so far. Alternatively a decision is needed on whether the substance ought to be rejected, or whether to continue testing. However, in principle, it is desirable that any food additive should be fully examined for all toxicological potentialities before its safety-in-use can be accepted (see also "Special Considerations", page 21).

a) Acute toxicity studies

The results of acute toxicity tests provide a basis for the classification of a substance in terms of its relative toxicity compared to the known acute toxicity of structurally similar and/or biologically related substances. Acute toxicity tests also furnish preliminary information on which to base the dosage range for feeding experiments of longer duration, by determining the dose levels which have or have not an effect on the experimental animal.

Furthermore, acute studies provide information about the target organs as determined by gross post-mortem examinations. They may provide useful information on the probable mode of action and evidence of the hazard to man to be expected in case of excessive exposure through accident, misuse or occupational handling.

The manner of administration of the substance should reflect the potential route of human exposure. In case of food additives the oral route is essential. The tests should be conducted over an observation period of at least 2 weeks and in more than one small laboratory species.

b) Genetic toxicity studies

Mutation refers to those changes in the genetic material of somatic or germ cells brought about spontaneously or by chemicals or radiations, whereby their successors differ in a permanent and heritable way from their predecessors.

Some types of mutation may not be manifested for many generations. The detection of chemicals that may be potential human mutagens has recently been a rapidly expanding field. This is because, apart from the serious implications for future generations if additional genetic diseases are added to the far from negligible current burden, there is evidence that somatic cell mutations, as opposed to germ cell mutations, may be associated with the development of certain types of cancer.

Damage to the genetic apparatus may be at the level of individual genes (gene mutations) or the interference may be a grosser type in which the structure of the chromosomes (structural chromosomal aberrations) or their number (numerical chromosomal aberrations) is altered. If the structural alteration is small and results in the deletion of one or a few genes, the net effect may be difficult to demonstrate visually and to distinguish from a gene mutation. A wide variety of procedures has been devised to test the ability of a chemical to induce these various kinds of mutations in organisms ranging from bacteria (prokaryotes), with the simplest arrangement of the deoxyribonucleic acid (DNA) molecule, to organisms, in which the DNA is arranged in a most complex association with proteins and enzyme systems (chromatin) to form the chromosomal system found in phyla ranging from fungi to insects and finally mammals (eukaryotes).

It is known that many chemicals possess mutagenic properties which present a potential hazard to future generations and thus there is a necessity to identify and limit the spread of chemicals with such properties into the human environment. It is therefore recommended that any new food additive should be investigated for mutagenic potential by testing procedures which cover both gene and chromosome damage, both in vitro and in vivo. In this way some evidence may be provided upon which a preliminary assessment of a possible mutagenic hazard can be based. If for any reason suspicion should arise about any food additive already in use, it should also be subjected to these test procedures. It is clearly impractical for any testing routine to cover the entire spectrum of genetic toxicity tests currently available. However, there is considerable and growing scientific evidence that the majority of potentially mutagenic chemicals can be detected by a combination of test procedures selected in such a way, that the hereditary machinery is tested systematically at increasing levels of complexity. A sequential approach also permits some flexibility in the choice of equivalent genetic targets.

A suggested battery of screening tests for carcinogenicity and mutagenicity investigates the following genetic endpoints:

i) The detection of gene mutations in bacteria both with and without the use of mammalian metabolic activation systems. This is incontestably the most widely validated system in the field of genetic toxicology and it is also believed to be the most sensitive. Once the activity of a chemical on the reversion DNA system in microorganisms has been clearly established, all further testing should be carried out in systems which utilize higher eukaryotes, preferably mammals.

ii) The determination of the ability to produce damage to the chromosomes of mammalian cells grown in vitro (metaphase analysis). This procedure has particular relevance in that human lymphocytes could be used.

iii) The testing of the ability to penetrate to, and interact with, genes in the complex arrangements of DNA in eukaryotic cells in vitro (fungi, yeasts or mammalian cells) or in vivo (Drosophila melanogaster or mice).

The bacterial genome is not only relatively simple compared to that of the eukaryotic cell but it is, particularly in specially constructed test organisms, readily accessible to chemicals. Hence suitable mammalian cell techniques based on detection of mutations at specific gene loci are employed. The mammalian cell grown in culture does not possess the metabolic capacity of the intact animal and thus it is necessary to conduct all in vitro tests with and without the provision of supplementary metabolic activation systems.

iv) The determination of mutagenic activity in in vivo tests in a mammal because in vitro systems necessarily fall far short of the metabolic possibilites in the intact mammal. A variety of tests is available at present e.g. metaphase analysis of bone marrow in the rodent, the micronucleus test in the rodent, a dominant lethal test in the rodent, which latter may also be carried out in conjunction with a multigeneration reproduction test.

v) If circumstances specifically demand it, it may be desirable, as an adjunct to this battery of screening tests for carcinogenicity and mutagenicity, to assay also for primary non-specific damage to DNA or stimulation of unscheduled DNA synthesis, to assay for cell transformation using appropriate in vitro cultured mammalian or human cell lines, to test for induction of heritable translocations and to test for somatic point mutations in rodents.

Positive evidence of binding to DNA, of the production of active metabolites in body fluids, and of cell transformation lends considerable support to regarding other evidence of mutagenicity as indicating the existence of a definite carcinogenic potential requiring verification by chronic testing in animals.

c) Metabolic, including pharmacokinetic studies

Metabolic and pharmacokinetic* data are vital characteristics of a compound and are needed for planning approaches to safety evaluation, for designing protocols, selecting the proper test species, defining the dose regimen, and for appropriate conduct of the studies. Until recently the principal justification for developing this information was the value of interspecies and interstrain comparison in order to select for toxicity tests those species and strains most closely resembling man in regard to the metabolic pathways of the compound. However in the case of food additives, in contradistinction to drugs, it is much less often possible to study human metabolism. Here studies in non-rodents and non-human primates may be useful and may provide an indication of the relative importance of possible metabolic pathways for a test compound; such studies are never a complete substitute for investigations in man but represent a practical approach, if human studies are not possible. Qualitative similarities regarding the nature of metabolites formed may not reflect similar rates of formation and pharmacokinetics in different species. Moreover, valid conclusions as to metabolism cannot be drawn from comparisons of results derived from tests carried out under different dosage regimens.

To provide a general overview of the fate of an ingested substance in the body, a number of relatively simple studies may be performed. Because metabolic differences are known to exist even among different strains of the same species an additional study to eliminate this possibility may be useful at this early stage of investigation. In vitro procedures such as investigation of acid, alkali or enzyme hydrolysis, and possibly the use of perfused organs may yield useful data. Possible interactions of the test compound with food components and likely changes of the test compound, while it is on or in the food, require consideration.

The test compound may undergo degradation in the gastro-intestinal tract, or may interact with substances present in the intestinal contents. Degradation may occur by physico-chemical means or by enzymes or by biodegradation through the action of intestinal bacteria. Under the latter circumstances individual variations may be noted within groups of animals as well as within different species. In vitro tests with gut contents or with cultures of isolated aerobic gut bacteria may be useful indicators of potential degradation, when the test compound is administered orally in vivo.

If there is stringent evidence that the compound is not absorbed after ingestion, further metabolic studies may not be needed, but non-absorption in one set of circumstances may change with alteration in the gut flora. The identification of a highly toxic metabolic product may render the parent compound unacceptable for use in food. Data indicating the physiological handling of the metabolites may be encouraging evidence of safety.

To check the variety, nature and range of metabolites formed, preparations from animal or human organs may be useful. Tests using isolated microsomes, liver cells, perfused organs, whole body or other forms of radio-autography, and the detection of the capacity of a compound to form chemically reactive metabolites provide insight into the toxic potentialities of the test compound, give clues to the understanding of species differences in toxicity, and contribute to the extrapolation of test results in animals to man.

For any given chemical there may be numerous and diverse pathways of biotransformation. Usually the effects of age, sex, diet, enzyme inducers and dose upon rates of formation, distribution and excretion of metabolites cannot be predicted.. Hence metabolic studies are conducted with radioisotope-labelled compounds to identify the main metabolites, their conjugated derivatives, the quantitative and temporal relationships of uptake, distribution, retention and elimination of radioactivity, and changes in the metabolic pattern with various doses of the test compound. Both untreated and "adapted" animals may have to be investigated.

*The term "pharmacokinetics" is used in these guidelines but alternative terms are "toxicokinetics" or "chemobiokinetics".

Comparative metabolism should be studied in those species and strains likely to be used for subchronic toxicity tests and dose effects should be investigated also in those species eventually to be utilized for long-term feeding experiments. It may be desirable for some metabolic work to be carried out in non-rodents e.g. dogs or non-human primates.

Ideally, pharmacokinetic information should be developed as soon as subchronic repeated administration tests are contemplated, but this depends on the availability of appropriate assay methods or of the radiolabelled compound. The objective is the gathering of data on the apparent volume of distribution and rates of absorption, distribution and elimination. Conventionally a single bolus dose is administered by gastric intubation and a simple balance study is carried out in blood, urine, faeces, bile and possibly cerebrospinal fluid at predetermined intervals over 96-120 hours. This provides a general view of the intake-output relationship of the compound.

More extensive investigations are needed to gauge the effects of very high doses, to characterize the major metabolites formed and their relative proportions, as well as to provide information on tissue distribution and tissue levels, target organs and the nature of the toxic mechanisms.

d) Subchronic studies

The primary objective of a subchronic study as part of the toxicological appraisal of a food additive is the characterisation of its physiological impact following repeated administration over a significant fraction of the life span of the test species. Subchronic studies also permit a judgement on the need for additional or more extended studies to delineate more clearly the toxicological profile of the substance under test. They are invaluable for determining the appropriate dosage levels for conducting chronic toxicity studies, because they provide information on the major toxic effects of the test compound, on dose-response relationships and on the reversibility of any phenomenon observed. Apart from giving an indication of the minimum dose causing any toxic effect, such studies point to the existence of species differences in the nature of the response and to the effects of environmental variables on the characteristics of the observed toxicity.

A major objective of subchronic studies is the establishment of the spectrum of toxicological effects of a compound, their nature and severity, in an animal species in which the metabolic pathways of the same or analogous substances are as similar as possible to those in man.

In many instances it has been possible to make a judgement with respect to the suitability of a substance as a component of the human environment on the basis of the results of subchronic studies. The extent to which subchronic tests can be relied upon to evaluate the safety of a substance depends on a number of factors such as chemical structure, the nature and extent of human exposure, and the character of the biological responses of animals to exaggerated dosage with the compound.

Conventional subchronic toxicity studies are usually limited to dietary exposure of two laboratory species for a period varying from 90 days to 1 year, generally representing 10% of the life span of the species selected. In the common laboratory rodents e.g. the rat, mouse and hamster, this period extends conventionally over 90 days. In the longer lived species, such as the dog, pig and subhuman primates, it may be extending over 1/2-2 years. One rodent and one non-rodent species should be employed. Traditionally the rat and the dog are used, but a second rodent species, the mouse, is frequently resorted to because of its availability and relatively short life span. In order to benefit from the existing data base and background information it is preferable to use commonly employed strains of rodents, unless knowledge of the comparative metabolism and toxic effects of the substance in man and other species dictates otherwise. Both sexes should be studied in order to detect differences due to sex hormone effects.

On the basis of acute toxicity data and on estimated or predicted intake levels under conditions of use, at least three dose levels excluding controls are selected in the hope of bracketing the "no observed adverse effect" level for the species under the specific conditions. At least one dose level should reveal an observable effect. It may be expedient to initiate the study with larger numbers of test levels and to discontinue those which prove too toxic after the first few weeks. During this period, significant deviations from control data in any observed parameter are considered "effects" observed. The degree of significance depends, of course, on the size of groups employed, the number of animals

exhibiting adverse signs, and their severity. The number and extent of the examinations made in such studies vary somewhat with the known properties of the compound, and the purpose of the test, but it is useful to establish a routine of careful clinical assessment.

One problem in the evaluation of observations during the terminal stages of a subchronic study is the degree of importance to be attached to reversible clinical and biochemical findings. Reversible changes may represent a biological response to stress. The stress in such cases being the adaptation to an unusual load on some metabolic pathway through which the animal attempts to mitigate the effects of the substance by accelerating an existing enzyme reaction, initiating a new one (enzyme induction), or overtaxing an excretory process. An example of a commonly seen reversible effect is the mild liver enlargement frequently observed after feeding certain compounds without any detectable alteration in the histological architecture of the organ. To investigate reversibility with a view to evaluating this phenomenon, some proportion of the survivors of a subchronic study should be continued on the basal control diet for up to 3 months after cessation of exposure and then examined for reversal of the adverse effects seen in those sacrificed earlier. Kinetic studies may assist in determining the appropriate exposure and reversibility periods.

e) Reproduction and teratogenicity studies

The purpose of reproduction studies is to provide information about the possible increase, in successive generations, in sensitivity to a substance, the effects on the fertility of male and female animals, the detection of any pre-, peri- and post-natal effects on the embryo, the foetus, and the young, including any teratogenic and mutagenic effects, and the discovery of peri- and post-natal effects on the mother.

The test should comprise at least two filial generations and the active substance is administered throughout the test at several dosage levels. Thus weanling animals are reared to maturity on the test diets, mated, and the F1a or F1b progeny carried through a complete maturation and reproductive cycle. The process is repeated for the F2a and F2b and where applicable for the F3a and F3b generation. In the course of these studies all the conventional behavioural and clinical observations are made, supplemented by data for reproductive efficiency and performance. In addition the usual indices are determined for conception and gestation, the number and size of litters, the weights of pups at birth, their viability, survival and growth through lactation. The study design should permit observations on fertility of the males.

The dose levels to which the animals are exposed should be similar to those used in the long-term studies. However, one of the dosages should be designed to exhibit the adverse effects to be expected from the subchronic oral toxicity studies. Laboratory rodents are to be preferred though other animal species merit consideration.

The induction of teratogenicity is one of the possible adverse effects on human reproduction during gestation. A teratogen may be defined as an agent that produces permanent structural or functional damage during embryogenesis, the net effect being a congenital malformation or organ malfunction which is usually, but not always, detectable at birth. Thus teratogenesis is a post-gametic phenomenon which theoretically could act at any time after the first cleavages of the implanted zygote. A mutagen, by contrast, acts on the gametes at some stage in their development from the germ cell stage and produces a heritable genetic alteration in these cells. Teratogens are usually considered to act during the stage of organogenesis, the classic example of a chemical teratogen being thalidomide. The detection of an environmental teratogen affecting man at present can be ascertained only by retrospective surveillance which is a difficult and unrewarding but necessary procedure. Prospective animal tests should therefore be designed to give some reasonable probability of inferring that a substance poses a potential teratogenic hazard to man. Unfortunately knowledge of the many factors that may underlie the induction of teratogenesis by a chemical in a human embryo is sparse. The choice of an animal model, and the actual test design, must be therefore largely empirical and to a considerable degree each substance tested must be considered as a separate experiment. At present there is no feasible alternative to studies carried out in a pregnant mammal.

All new food additives, and those in current use if suspicion arises, should be tested for teratogenicity in at least two species. The practical possibilities are the mouse, rat, or rabbit; in certain circumstances other species have been used, e.g. the hamster and the guinea pig. It is essential that there should be extensive background knowledge of the

strains of animals chosen. The accumulated data on all the defects observed in the past in untreated animals of the chosen strain form the historical control group. This information, together with details of the particular species and strains used in the studies must be presented with the results. It is desirable that one of the species chosen is the same as that used in the chronic toxicity studies. The design and conduct of teratological testing is a matter that only well-qualified and experienced toxicologists should undertake. They will be aware of controversial matters, the possibility of controlling the many experimental variables in the actual circumstances of the trial, of the protocol that is most suitable for the individual chemicals under test, and that no one test system can be specified, that will indicate with absolute certainty whether a chemical is potentially teratogenic for man. The available methods are empirical and thus only general principles can be usefully indicated. Tests should be conducted with scrupulous attention to the details of the particular test design that is judged to be most suitable in the circumstances.

As an alternative to the separate setting up of teratological studies involving exposure only during fixed periods of organogenesis, it has been suggested that a portion of the pregnant rats of one of the generations of a multigeneration study are used for teratological investigations.

The data supplied by this modified teratological phase are quite adequate in terms of the numbers and types of observations. They differ, however, from most published procedures in that exposure of the dam is continuous from the moment of insemination to the end of pregnancy. In cases where the number of implantations is reduced, an increased incidence of abnormalities is observed, the relationships between dose and severity or frequency of effect appears significant, or it is desired to establish the embryonic stage at which the conceptus is most sensitive, appropriate teratological testing in greater depth may be required.

It should be remembered that, although a dose-response relationship exists, it may not be easy to demonstrate this clearly in teratological studies, since the intervention of maternal metabolism and the protection afforded by the placental barrier are important complicating factors.

f) Chronic toxicity and carcinogenicity studies

The objective of chronic testing in animals, by exposure for most of its life span to the test substance by an appropriate route and at appropriate dosage, is the assessment of potential toxicity as a result of long-term, low-level exposure. Such toxicity may not be evident in subchronic studies in test animals and comprises cancer, certain irreversible and progressive forms of toxicity, and toxicities dependent upon age-related sensitivities of certain tissues. This type of experiment is subject to many potential variables which are difficult to control and which may influence the final interpretation of an experiment. Chronic testing, despite its relative insensitivity and procedural limitations, is however at present the only appropriate method to evaluate the potential risk of progressive irreversible toxicity or of carcinogenicity.

The mechanisms of carcinogenesis are not at present clearly understood. Physical, chemical, biochemical, hormonal, nutritional, genetic, viral and other factors may be involved. Direct clinical evidence and epidemiological studies in man can make a considerable contribution to the determination of chronic toxic hazards, whilst human evidence of carcinogenicity would militate against the acceptability of a substance as a food additive. The role of immunological reactions in relation to carcinogenesis is not fully understood at present but should be considered in hazard evaluation where justified by appropriate findings.

For food additives it is recommended that combined protocols be used for studying chronic toxicity and carcinogenicity in the same experiment. Most of the procedures for carrying out such chronic studies are relatively standardized but several areas are still considered controversial. The selection of test species is limited by many practical considerations, although the animal model should be biologically appropriate for the toxicological assessment of the possible human risk. This implies that metabolism and pharmacokinetics of the test substance in the species and strains chosen should mimic those in man as closely as possible. For food additives both mice and rats are the traditional species employed, because of the relatively short life span, size, cost and extensive experience and availability of information on their biological characteristics. Both species have to be used in most circumstances unless specific considerations dictate otherwise. Dogs and non-human

primates are usually unacceptable because of their relatively long life spans, unless the nature of the toxicity or the procedures required necessitate the use of large species. Historical information should be available on the specific pathological alterations in the species and strains studied. The use of inbred versus outbred animals remains controversial.

Consideration of the experimental designs which have been suggested favours the use of adequately sized control and test groups. If adequate historical control animal data are available, one contemporary control group for each sex and species suffices. If such data are not available, larger control populations may be necessary. The use of separate control groups, one housed together with the test groups and the other in a separate control room, remains controversial. Similarly, design variations which include the provision of additional animals for interim sacrifices or the setting up of an experiment with greatly increased numbers of test animals exposed to the lowest doses of test substance, are applicable only if cogent reasons exist for the use of such special experimental designs. It is suggested that three dose levels apart from controls should be tested. The highest level chosen should be the one which in subchronic tests induces no overt toxicity or toxic manifestations, and is predicted not to shorten the life span of the animals except as a result of neoplastic development. Furthermore, it should not retard the weight gain by more than 10% as compared to control animals, and should take into account metabolic and pharmacokinetic data.

For food additives, which are essentially non-toxic chemicals, it may prove impossible to identify a dose level which is toxic, or the administration at the maximum tolerated level may entail such major alterations to the composition of the diet, that a meaningful experiment is impossible. The administration of doses of any compound at dose levels in excess of 5% of the diet may cause non-specific chronic toxic effects and these may in turn influence tumour incidence non-specifically. In such cases the highest dose level should generally be set at 5% of the diet.

If the design of the experiment incorporates <u>in utero</u> exposure, not routinely but only if specific considerations demand it, then the highest level tested should not be detrimental to conception rates, foetal or neonatal survival or post-natal development. Such complex designs involving exposure before birth are useful for investigation of the possibility of transplacental carcinogenesis. The use of newborn animals because of their possible greater susceptibility to carcinogens is complicated by the concomitantly increased difficulty in interpreting the results of tests. Newborn animals are difficult to expose to accurately measured doses except by the parenteral route and some species are prone to respond under these circumstances with an increased tumour incidence even if agents are used which do not induce tumours in adult animals.

Exposure to a test substance is currently required for the major portion of the life span of the species used. Provided adequate husbandry is practised, a 24 months post-natal exposure period is generally considered adequate for both rats and mice. Ideally, all or most animals will survive for this period, at which time terminal sacrifice should be performed. Exceptionally, provided that dosing is continued for at least 18 months in mice and 2 years in rats, there may be advantages in stopping exposure thereafter provided observation of the animals is continued until sacrifice.

The results of a carcinogenicity test in mice would not normally be regarded as negative unless there was a 75% to 80% survival of the animals in each test group for 18 months from the start of dosing. A similar survival rate over 2 years should be expected in rats.

The optimum point of termination of the experiment may be influenced by the increasing incidence of spontaneous disease, including malignant disease, with the age of the animals. The duration will thus vary and sacrifice may take place at the planned endpoint of the experiment, or there may be advantages in continuing the experiment. Some protocols suggest to effect terminal sacrifice when 20% of the second highest test group survives. Although survival up to 30 months or more might permit greater expression of any carcinogenic effect, spontaneous tumour rates also increase markedly in advanced age and this effect may mask a positive effect. There is little available data to suggest that evidence of carcinogenic effect in mice or rats first appears only after 24 months of chronic exposure.

g) Other toxicological investigations

Progress in toxicology has led to the exploration of new avenues which may be valuable in the overall aim of making toxicological evaluations of safety more accurate and comprehensive. Rapid advances have been made in such areas as immunotoxicology and behavioural toxicology. New procedures are constantly being developed and the problems of interpreting animal observations in these fields in terms of human hazard are being gradually overcome. The state of the art in these areas of toxicology has not yet reached the stage where generally acceptable protocols can be recommended. This is not to be interpreted as a judgement that experimentation directed towards exploring the potential for causing adverse effects in these fields has no practical utility. Caution has to be exercised, however, in any extrapolation of these special findings in laboratory animals to the human situation.

In drawing up these general guidelines the Committee has been attempting to give an overview of the state of the art in the toxicological investigation of food additives as it exists at present. It is accepted fully that this situation is subject to change in the light of new developments and that these guidelines may be revised in the future to take account of advances in toxicology. Moreover there must be freedom for the toxicologist to modify procedures or use alternatives. If this is being done, however, valid reasons for such changes must be presented for appraisal.

INTERPRETATION AND EVALUATION OF TEST RESULTS

Because the safety of a food additive can never be proven absolutely, the word "safe" has to be interpreted in some circumstances as meaning a socially acceptable potential risk under the existing or predicted conditions of consumption or exposure. When toxic or other undesirable effects are found in test procedures, it is almost always at exaggerated dose levels. It then becomes necessary to estimate the probability of a similar response at dose levels encountered under normal conditions of consumption. For this purpose an estimate of the total dietary intake of the food additive from all sources is essential. Such data may be obtained by extensive total diet studies of various sample populations and by diet analyses to determine as accurately as possible the pattern of technological use of the food additive. Recourse may be had to national diet surveys for estimates of consumption levels of individual components of the diet both for average and high consumers.

When toxicological and other relevant data are submitted for evaluation the first aspect to be considered is the manner in which they have been obtained, their completeness and relevance to the assessment, the possibility of checking their validity and whether the laboratory circumstances were acceptable in terms of modern laboratory practice. In evaluating the results of adequately performed toxicological studies attention should be directed initially to the nature of the observed biological activity. Furthermore, it is important to decide whether any observed effects are reversible or irreversible, whether they are cumulative, and whether they are of a purely functional or purely morphological nature or both. Another consideration concerns the predictive value of the test performed and whether any dose-effect and dose-response relationships can be established.

The concept of acceptable daily intake

The aim of the toxicological investigation of any food additive has been to establish the safety-in-use of the additive. In most cases this will result in the establishment of an acceptable daily intake (ADI) for man. The latter was defined originally by the Joint FAO/WHO Expert Committee on Food Additives as representing the average amount of a substance, expressed in mg/kg body weight, which can be ingested daily in food throughout the human life without any obvious harm to health on the basis of all known facts at the time of evaluation.

Where an ADI is expressed as "no upper limit specified", this means that, on the basis of the available toxicological, biological, chemical and clinical data, the total daily intake of the substance, arising from its use or uses at the levels necessary to achieve the desired technological effect and from the acceptable background in food, will not in any of these circumstances represent a hazard to health. For this reason the establishment of a numerical limit for the ADI is not considered necessary for these substances.

In certain circumstances it is not possible to establish a numerical value for the ADI from the available toxicological data by the usual classical procedures. Nevertheless appraisal of the toxicological information allows the conclusion to be drawn that the use of the substance for a limited period would present no hazard to the health of the consumer and that there are no grounds for suspecting that the substance is toxic. To cover this situation the concept of a "temporary ADI" has been developed, subject to the following conditions:

i) the available biochemical and toxicological data, even if incomplete, are nevertheless sufficiently extensive to exclude the possibility of any risk to health of the consumer during the period of validity of the temporary ADI;

ii) a higher safety factor must be used in establishing the temporary ADI;

iii) the period of validity of the temporary ADI is limited to that required for completion of any toxicological studies in progress, this is generally 3-5 years;

iv) the nature of the research to be undertaken and of the additional information required is clearly stated and the reasons for requesting their provision fully explained.

In the end this course of action will permit an estimate to be given of the ADI of the substance based on complete toxicological data.

The concept of "no effect level" (NEL)

The objective of quantitative toxicological studies is the determination of the level of a substance that can be included in the diet of experimental animals without toxic effects. However, in designing toxicological studies it is necessary to use deliberately doses producing an effect in order to determine target organs and give some indication of the nature of the toxic effect. With certain substances even the highest level that can be incorporated in a diet fails to produce any observable effects. However, some food additives do exert toxic effects when fed at high levels. For these the maximum no-effect level should be determined in the most appropriate animal species and be based on the most pertinent criteria of toxicity.

The idea of a NEL derives from the classical pharmacological concept of a dose-effect relationship controlling the effects of chemical agents on biological systems. As a corollary there must exist a threshold dose below which no effect occurs. In the past toxicology has relied on standard experimental protocols. Chemicals were administered to groups of animals, and the development and health of the animals were monitored by known clinical laboratory and pathological techniques. In modern toxicology special experiments are designed for assessing a particular effect quantitatively under highly standardized conditions by establishing a dose-effect curve. The observed biological changes are related to blood and tissue levels of the compound or its active metabolites. Such experimental procedures encompass the effects of chemicals on a large number of enzymatic and metabolic processes and molecular biological events. These effects are often not accompanied by overt pathological or histopathological manifestations, neither need they be associated with measurable alterations in organ functions. Emphasis has shifted therefore from the determination of a NEL to a detailed analysis of the full spectrum of biological events invariably induced when a foreign substance enters a living organism.

A consequence of this new approach is the difficulty in determining a NEL in the classical sense of the word. The observational limits are determined largely by the sophistication of the investigator's tools. There may be orders of magnitude between the NEL determined by light microscopy, electron microscopy and highly sensitive biochemical prodedures. Clearly any NEL determined by these nonclassical toxicological procedures cannot be used in the same way for establishing ADIs as the NEL determined by the much cruder and less sensitive standard toxicological methods.

It has been pointed out in many discussions that a variety of effects may occur in toxicological experiments that are deemed not to be of toxicological significance. If such effects are purely attributable to normal physiological adjustment to metabolic overload, they may be disregarded when establishing a NEL. Many of the subtle effects detectable by sophisticated methodology may fall into this category of physiological adaptation, but often precise proof may be difficult. In many instances even these more esoteric effects require consideration and may stimulate research which will assist in establishing safe levels of use of a substance.

The assignment of a NEL for establishment of an ADI should be based in principle on the outcome of conventional methodology in subchronic and chronic animal studies conducted according to generally accepted "classical" toxicological protocols. However, evaluation of the full spectrum of biological effects of substances should be encouraged using the appropriate experimental techniques. The results obtained should be ranked by comparison with other compounds, whose toxic potential in man is better understood, thus permitting a sound evaluation of the toxicological significance of the data obtained and the establishment of an ADI supported by more relevant biological data than would be available from: "classical" methodology.

Margin of safety

At the completion of toxicological studies of food additives in experimental animals a NEL may be determined for laboratory species, expressed in mg/kg in the diet. Suitable conversion factors allow the establishment of the corresponding laboratory animal intake in mg/kg body weight per day, taking into account the average weight of the animals and the mean daily food consumption.

In extrapolating this laboratory animal intake to man in the form of an acceptable daily intake (ADI) for man some safety factor is necessary. This safety factor includes contributions specifically designed to cover differences in species sensitivity; the heterogeneity of the exposed human population with regard to health, pregnancy, physiological status and nutrition; synergistic or antagonistic action among food additives and with other components of food; as well as age differences between exposed individuals and the known variability with age in susceptibility to the potential adverse effects of an ingested foreign substance.

Classically an arbitrary safety factor of 100 has been widely accepted but it would be unreasonable to apply this factor rigidly in all circumstances. There are circumstances justifying the use of a lower safety factor, for example, if the food additive is an essential nutrient, a normal constituent of the body or metabolises into substances with similar characteristics. Similar considerations would apply if the criteria for establishing a NEL fail to disregard effects of doubtful toxicological significance, or if valid human data are available.

On the other hand there may be appropriate reasons for increasing the safety factor. Examples of such circumstances, although these are unlikely to apply to acceptable food additives, are the particularly serious significance of observed toxic effects following deliberate overdosage, e.g. deleterious effects on major organs such as kidney, liver or the central nervous system, or the existence of a teratogenic or mutagenic potential. Other situations are the need to maintain the continued use of the food additive even if the available toxicological information is not fully adequate in all respects. Similar considerations may apply, if exposure to the food additive shows wide variations in intake by critical population groups or if the food additive is proposed for use in food items consumed preferentially by sensitive population groups, such as children. Evidence for synergistic effects may also justify an increase of the safety factor. There are a few special situations in which critical groups may display excessive susceptibility to specific toxic effects of a foreign substance to which they are exposed. In these cases it may be prudent among other measures, to use safety factors higher than those normally employed in establishing ADIs.

Applications of the ADI concept

The ADI concept, in its classical and modified forms, has proved to be of immeasurable benefit since its initial development for the evaluation of the health hazards for man of almost all environmental substances. It represented the best practical approach for establishing the acceptability of intentional and unintentional food additives, pesticide residues, food contaminants, etc., and offered a means of achieving some uniformity of approach in the regulatory control of these substances. It has served and continues to serve its purpose well in the great majority of cases, where it is being applied. Nevertheless certain difficulties in applying the ADI concept to all situations have led to suggestions for alternative approaches.

In establishing numerical values for ADIs it must be remembered that these are intended as a guide and are themselves based on experimental data showing a large variability. They should not be regarded as mathematical concepts indicating a sharp demarcation between safety and health hazard. ADIs are calculated from animal experiments by the use of largely arbitrary safety factors and thus contain a safety margin such that in expert judgement there need be no undue concern when the ADIs are occasionally or slightly exceeded. These circumstances therefore do not constitute a hazard to the health of the consumer. Minor fluctuations in daily intake of a food additive above and below the ADI are usually self-compensating if averaged over long periods. However, if the ADI appears from estimations of intake to be exceeded for long periods, expert reassessment of the situation may be required. Even then the situation will differ for food additives with practically no toxic potential and for those with some evidence of a toxic response in laboratory investigations. In the latter case reevaluation would have to be more searching in depth and extent.

Extrapolation of results in animals to man

Undoubtedly the most satisfactory evidence on which to base an assessment of human hazard would be adequate human studies. The difficulties and limitations arising in the pursuit of this approach only rarely permit evaluation by this procedure. Reliance has therefore to be placed on animal experiments together with the assumption, that man reacts essentially like the most sensitive species tested unless there is proof to the contrary. The more comparable the selected test species is to man, the more certain extrapolation would be. Unfortunately, this ideal can rarely be achieved and cognisance must be taken of the variability in response and sensitivity of different animal species and strains selected for laboratory studies. Sensitive and relevant criteria of toxicity are required for extrapolation of the results of animal experimentation to man.

A number of important factors need to be considered in this procedure. These are:

1. The uncertainties inherent in quantitative animal data, for instance the failure to observe an adverse effect because of the limited number of animals which can be used in toxicological studies. Other uncertainties arise from the failure to detect human toxic responses for which no adequate animal experimental models exist.

2. The choice of functional and morphological criteria for determining the NEL which are governed by the scope of the experiment.

3. The difference in body size between experimental animals and man. Man has also a lower metabolic rate per unit body weight compared to small laboratory animals and may differ in his capacity for biotransformation and pharmacokinetic handling of foreign substances.

4. The species specificity in reacting to the presence of a foreign substance in the body resulting in qualitative and quantitative differences in toxic manifestations observed in man and laboratory animals.

5. The variability of the reactions within the human population arising from differences in genetic disposition, age, nutritional status, health status, environmental circumstances, climate, etc. These variations are largely controlled in the homogenous animal population used for toxicological testing.

6. The possible interaction of food additives with other substances simultaneously ingested by man.

Special considerations apply to substances shown to have carcinogenic and/or mutagenic effects. These may act either directly or after metabolic activation with appropriate macromolecular targets in the affected body cells and constitute primary genotoxic compounds for which thresholds probably do not exist. There are also other compounds with similar end effects which do not act by these direct mechanisms and these constitute epigenetic carcinogens or mutagens. The latter substances have different properties from genotoxic carcinogens and for these a threshold may exist. In principle substances shown by adequate bioassays to be potentially genotoxic in animals should not be added to the human food supply. This consideration may not apply as rigidly to substances acting by epigenetic mechanisms.

For substances with purely heritable mutagenic potential, which do not produce any positive results in adequately performed carcinogenicity bioassays, there is at present no possibility of making quantitative risk assessments in terms of the induction of increased mutation frequencies in man. Here only broad categorization of mutagenic activity can be achieved, based on appropriate combinations of the outcome of *in vitro* and *in vivo* genetic toxicity tests for different genetic endpoints. Interpretation of these results on their own in terms of human mutagenic hazard is not possible at present and any evaluation has to be made on the basis of all available toxicological data. Each compound has therefore to be considered on its merits.

Special considerations

For certain classes of food additives such as flavouring substances, processing aids, e.g. filtering aids, and food packaging components, the extent of the toxicological examinations described above may need to be modified appropriately. As an example, in the case of flavouring agents, different considerations may apply when evaluating their safety-in-use. Many of these substances occur in natural food products or are used in minute concentrations in a few foods only. On the other hand, many have not yet been full characterised. As these substances are very numerous, priorities need to be established regarding the substances to be evaluated, giving at the same time due consideration to the extent of the toxicological examination required. It must be remembered, however, that no toxicological evaluation can be made in the complete absence of information (see also page 8). An example of a possible approach is the one used by the Council of Europe. The 21st Report of JECFA gives an example for the selection of criteria for priorities.

For components of food packaging materials guidelines have already been published[1].

In the case of processing aids the requirements for toxicological examination may be restricted, if the substances are clearly defined and specified, if they have a satisfactory degree of purity, if they are essentially removed during processing without leaving residues or by-products or substances resulting from reaction with food components. In other circumstances their potential health hazards have to be evaluated, depending on the nature of the substance and of any residues appearing in the processed food.

ANNEX PRO MEMORIA

It is proposed, at a later date, to include as an Annex more detailed protocols for conducting toxicological investigations. These are at present being established in international fora and their applicability for the toxicological examination of food additives will be considered by the Scientific Committee for Food. The Committee intends to propose minimum requirements rather than detailed test procedures.

Similarly the Committee will be considering the proposals for good laboratory practice at present being elaborated within the EEC and make appropriate suggestions for application in the field of food additives.

[1] Reports of the Scientific Committee for Food, Third Series, 1977.

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The Scientific Committee for Food was established by Commission Decision 74/234/EEC of 16 April 1974 (OJ L 136 of 20.5.1974, page 1) to advise it on many problems relating to the protection of the health and safety of persons arising from the consumption of food, and in particular the composition of food, processes which are liable to modify food, the use of food additives and other processing aids as well as the presence of contaminants.

The Members are independent persons, highly qualified in the fields associated with medicine, nutrition, toxicology, biology, chemistry, or other similar disciplines.

The present report contains general principles applied by the Committee in advising on health questions linked to the approval of the use of additives in food, and recommendations on the kind of toxicological data and technical information to be provided to the Committee for evaluation.

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