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**EVALUATION OF
THE CARCINOGENIC HAZARDS
OF FOOD ADDITIVES**

**Fifth Report
of the Joint FAO/WHO Expert Committee
on Food Additives**

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JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Geneva, 12-19 December 1960

Members :

- Dr Eldon M. Boyd, Professor of Pharmacology, Queen's University, Kingston, Ontario, Canada
- Professor E. Boyland, Chester Beatty Research Institute, Fulham Road, London, England
- Mr H. Cheftel, Directeur du Laboratoire de Recherches, Etablissements Carnaud-Basse Indre, 71 av. Edouard Vaillant, Billancourt (Seine), France
- Professor H. Druckrey, Laboratorium der Chirurgische Universitätsklinik, Hugstetterstrasse 55, Freiburg-im-Breisgau, Germany
- Professor A. C. Frazer, Department of Medical Biochemistry and Pharmacology, University of Birmingham, England (*Chairman*)
- Mr H. van Genderen, Chief, Laboratory of Pharmacology and Toxicology, National Institute of Public Health, Utrecht, Netherlands (*Vice-Chairman*)
- Dr J. A. Miller, Professor of Oncology, The McArdle Memorial Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin, USA
- Dr B. L. Oser, Food and Drug Research Laboratories, Inc., Maspeth 78, New York City, USA
- Professor L. Shabad, Deputy Director, Institute of Experimental and Clinical Oncology of the Academy of Medical Sciences of the USSR, Moscow
- Dr P. Shubik, Professor of Oncology, Chicago Medical School, Chicago 8, Illinois, USA
- Dr A. Tannenbaum, Department of Cancer Research, Michael Reese Hospital and Medical Centre, Chicago 16, Illinois, USA
- Professor R. C. J. Truhaut, Professeur de Toxicologie, Faculté de Pharmacie, Université de Paris, et Membre du Conseil Supérieur d'Hygiène publique de France, France

Observer (invited by FAO) :

- Dr P. E. Johnson, Executive Secretary, Food Protection Committee, National Research Council, 2101 Constitution Avenue, Washington 25, D.C., USA

Secretariat :

- Dr M. G. Allmark, Scientist (Food Additives), Nutrition, WHO (*Joint Secretary*)
- Dr A. G. van Veen, Chief, Food Processing and Preparation Branch, Nutrition Division, FAO (*Joint Secretary*)
- Dr C. Agthe, Scientist (Food Additives), Nutrition, WHO
- Dr R. C. Burgess, Chief, Nutrition, WHO
- Dr L. Verhoestraete, Director, Division of Health Protection and Promotion, WHO

EVALUATION OF THE CARCINOGENIC HAZARDS OF FOOD ADDITIVES

**Fifth Report
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INTRODUCTION

The Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 12 to 19 December 1960. The meeting was opened by Dr P. N. Kaul, Assistant Director-General, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Professor A. C. Frazer and Mr H. van Genderen were unanimously elected respectively Chairman and Vice-Chairman of the meeting. The Committee consisted of twelve members invited by FAO and WHO. An observer from the Food Protection Committee, National Research Council, USA, invited by FAO also attended.

As a result of the recommendations of the Joint FAO/WHO Conference on Food Additives held in September 1955,¹ four meetings of the Joint Expert Committee have been held and reports have been issued on "General Principles Governing the Use of Food Additives: First Report",² "Procedures for the Testing of Intentional Food Additives to Establish their Safety for Use: Second Report",³ "Specifications for Identity and Purity of Food Additives (Antimicrobial Preservatives and Antioxidants)",⁴ and "Specifications for Identity and Purity of Food Additives (Food Colours)".⁵

This meeting was convened on a recommendation of the Joint FAO/WHO Expert Committee on Food Additives meeting in Geneva in June 1957, that FAO and WHO should refer the problem of the possible carcinogenic action of food additives to an appropriate group of experts. This

¹ *FAO Nutrition Meetings Report Series*, 1956, No. 11; *Wld Hlth Org. techn. Rep. Ser.*, 1956, 107

² *FAO Nutrition Meetings Report Series*, 1957, No. 15; *Wld Hlth Org. techn. Rep. Ser.*, 1957, 129

³ *FAO Nutrition Meetings Report Series*, 1958, No. 17; *Wld Hlth Org. techn. Rep. Ser.*, 1958, 144

⁴ Joint FAO/WHO Expert Committee on Food Additives (1958) *Third report*, Geneva (Unpublished working document WHO/Food Add./15)

⁵ Joint FAO/WHO Expert Committee on Food Additives (1959) *Fourth report*, Geneva (Unpublished working document WHO/Food Add./17)

report is the result of the deliberations of the group and was adopted unanimously.

Terms of reference

In the letters of invitation the Directors-General of FAO and WHO stated that, in accordance with the recommendations referred to above, the Committee should consider the problem of the evaluation of the carcinogenic hazards of food additives. The Committee was of the opinion that, whereas at former meetings it had considered mainly intentional food additives, it would be appropriate on this occasion to include in its discussions food contaminants and substances occurring naturally in food.

SCIENTIFIC BACKGROUND

Several reports on or related to the evaluation of the carcinogenic risk arising from food contamination or from the use of food additives have been published during the last few years.¹⁻⁵ Examination of these documents reveals certain general considerations that can be regarded as providing a basis for discussion of the problems concerned with the evaluation of the carcinogenic risk. These are in broad agreement with the general principles laid down in the second report of the Joint FAO/WHO Expert Committee on Food Additives, 1958.⁶

The Committee unanimously adopts the following general basic principles :

(1) Food additives should be used for the benefit of the consumer ; they should not be permitted for any deceptive or misleading purpose.

(2) The use of certain food additives is justified since the present position with respect to world food supplies makes it imperative that good technological procedures should be developed and used to a maximal extent.

¹ International Union against Cancer (1957) *Resolutions of the First Symposium, Rome, 11-15 August 1956* ; (1961) *Recommendations of the Second Symposium, Tokyo, 9-13 October 1960* (mimeographed documents)

² United States of America, National Research Council, Food Protection Committee (1959) *Report*, Washington

³ United States of America, President's Science Advisory Committee, Panel on Food Additives (1960) *Report*, Washington

⁴ Permanent European Committee for Research on the Protection of the Population against Chronic Toxic Hazards (1954) *First report*, Bad Godesberg ; (1957) Report of the 3rd Meeting, Ascona. *Mitt. Lebensm. Hyg. Bern*, **48**, Heft 4 ; (1958) *Report of the 4th Meeting, Montecatini, Rome*

⁵ England and Wales, Ministry of Health, Committee on Medical and Nutritional Aspects of Food Policy (1960) Carcinogenic risks in food additives and pesticides. *Monthly Bull. Minist. Hlth Lab. Serv.*, July

⁶ *FAO Nutrition Meetings Report Series*, 1958, No. 17 ; *Wld Hlth Org. techn. Rep. Ser.*, 1958, **144**

(3) Food materials and all chemicals and processes at present in use for the preparation and distribution of food should be subjected to adequate examination to ensure minimum risk in use, if this has not already been done, or unless previous knowledge indicates that this is unnecessary.

(4) All chemicals or processes proposed in the future should be subjected to adequate examination to minimize risk before being accepted for use.

(5) The presence of carcinogenic substances in food might be a significant factor in the occurrence of what is considered to be spontaneous cancer in man and animals.

(6) Since dose-response relationships have been demonstrated in the case of carcinogenic agents, the reduction of carcinogenic substances in food to the lowest practicable level may be one of the effective measures towards cancer prevention.

(7) Many factors may influence dose-response in carcinogenesis. Their complexities are such that it is agreed that no assuredly safe level for carcinogens in human food can be determined from experimental findings at the present time.

(8) The elimination, or at least reduction to a minimum, of all proved carcinogenic substances in the diet of man and of animals used as human food is a worthwhile objective.

In recent years a number of food additives or food contaminants have been suspected of being carcinogenic. As a practical approach to the problems arising in this field, the Committee has reviewed the evidence available and the action taken with regard to a number of these substances. Many points of interest were revealed, of which the most important are :

(1) The apparent lack of information on the toxicity or potential carcinogenicity of many food additives.

(2) The inadequacy of the design, execution and interpretation of some experiments or of reported information in some of the publications in this field and the frequent lack of corroborative evidence.

(3) The need for detailed pathological evaluation of any lesion observed in experimental studies.

(4) The difficulties arising in interpretation of local sarcoma formation at the site of injection.

(5) The difficulty of completely excluding at the present time carcinogenic contaminants from food, from processes used in the preparation of food, and from substances coming into contact with food.

(6) The possibility that some natural constituents of the diet or even an essential nutrient, such as selenium, may constitute a carcinogenic risk. Clearly these substances cannot be completely excluded from the diet.

(7) The difficulty of carrying out and interpreting epidemiological studies.

(8) The wide variety of food colours, many of which do not appear to have been adequately tested, included in the "permitted" lists of different countries.

(9) The necessity for separate assessment of the carcinogenic risk for each individual substance.

The information collected seemed to be of sufficient interest to justify inclusion as Annex 1 to this report (see page 24). In the time available to the Committee, however, it has not been possible to make this annex as critical and exhaustive as might be desired. The Committee would like to stress, moreover, that they have made no attempt to evaluate the evidence presented. The material only provides a means of obtaining a practical insight into some of the actual problems encountered in this field.

TESTING PROCEDURES

Introduction

It is evident from a review of the literature that tests on experimental animals cannot provide irrefutable proof of the safety or carcinogenicity of a substance for the human species. However, it is at least reassuring that the known carcinogenic activities of certain chemicals in man are similar in many ways to those found in experimental animals. Hence it is only prudent to determine, so far as it is practicable, the carcinogenicity in experimental animals of substances used as food additives or occurring as contaminants. The results of such tests should determine to a considerable degree whether or not these substances should be used in the human diet. It is therefore necessary to formulate practical procedures for the determination of possible carcinogenicity within the limits of our present knowledge.

It is desirable that all food additives and contaminants be fully investigated. It must be recognized, however, that there are many substances which require study, and that some permitted lists contain apparently untested substances. The Committee also recognizes that the available facilities and experienced personnel for carrying out such studies are limited and may remain so for an unpredictable time. Therefore, the proposed tests should be relatively simple and no more time-consuming than necessary to facilitate the testing of as many food additives as possible within a reasonable period of time.

As has been emphasized in the second report of the Joint FAO/WHO Expert Committee on Food Additives (Procedures for the Testing of

Intentional Food Additives to Establish their Safety for Use),¹ the details of tests of food additives for potential carcinogenicity are the responsibility of the scientist. Nevertheless, some general and special recommendations may be given to make the investigations as informative as possible and acceptable internationally.

Scope of tests required

Several factors must be taken into account in deciding the scope of the tests required in the case of any particular substance, namely, the nature of the substance, impurities present, the proposed use, the particular food involved, the amount which is likely to be eaten, and the age and physical condition of the main consumers. Such considerations would allow for reasonable priorities in testing to be established and thus provide broader safeguards in the shortest possible time.

The minimum safeguard

The Committee has adopted the view that the minimum safeguard must be an investigation of the tumour incidence in a chronic toxicity test as set forth in general terms in the second report of the Joint FAO/WHO Expert Committee on Food Additives. In the opinion of the present Committee this should involve the study of an adequate number of animals of two species (e.g., rats and mice) subjected to the feeding of a suitable dose range of the substance under question for the lifetime of the animals. Where additional safeguards are considered necessary, because of the nature of the food additive or of its proposed use, further tests such as the use of a suitable parenteral route of administration, or studies in other species of animal, are recommended.

General considerations

In all tests on experimental animals, regardless of the route of administration, certain factors require attention. These are :

Identity and purity of material under test

The remarks on chemical and physical specifications in the second report of the Joint FAO/WHO Expert Committee on Food Additives also apply to special studies for carcinogenic activity of food additives. Important aspects are that samples to be tested should be representative of the composition of the material intended for human use, and that the chemical and physical properties used in identification of the samples (e.g., absorption spectra or chromatographic analyses) be reported in detail.

¹ *FAO Nutrition Meetings Report Series*, 1958, No. 17; *Wld Hlth Org. techn. Rep. Ser.*, 1958, **144**

It is desirable to obtain and preserve one sample for the complete test, but if the material is unstable, or if, for other reasons, it is necessary to use samples from different batches, then each fresh supply should be carefully checked for identity. Sufficient information on vapour pressure and stability should be available to decide whether or not the material can be administered to the animals by mixing it into the diet or into the drinking-water, and how frequently such mixtures should be prepared.

The identity of samples to be used for the testing of food contaminants presents difficulties, particularly when the exact composition of the contaminant is not known. For instance, some pesticide residues may be present mainly as break-down or reaction products. In preparing samples for testing, such possible changes should be taken into account. In special cases it may be necessary to test the treated food product as a whole, instead of the original pesticide or its known reaction products.

Animals to be employed, and duration of experiments

Both sexes of each of at least two species of animals should be used in the tests throughout their life span. In most cases these species would be rats and mice. Hamsters or dogs might be suitable, but guinea-pigs, for example, appear to be resistant to some known carcinogens. The use of dogs in carcinogenicity tests has disadvantages. Because of the expense of maintenance it is difficult to use a sufficient number to detect a low incidence of cancer, and the life span of this animal is 12-15 years.

The animals used should be bred in the laboratory in which the tests are carried out. The characteristics of the colony, including sensitivity to carcinogens, should be known, and the incidence of spontaneous tumours should be recorded. Out-bred or random-bred animals are generally acceptable. If pure strains are used then at least two strains should be employed. There are advantages in using F_1 hybrids of two pure strains of animals.¹

The treatment should begin when the animals are young—in the case of rodents, soon after weaning. Animals should be kept under good conditions and should be as free as possible from parasites or infectious diseases, especially those that may shorten the life span or may lead to tumour production.

Number of animals

The number of animals in each group should be sufficient to yield statistically reliable results. For example, where no tumours appear in the control group there must be at least 4 tumours in the experimental

¹ Boyland, E. (1958) *Brit. med. Bull.* **14**, 93

group for the result to be considered significant at a P value of 0.025 (see the following tabulation, derived from Boyland¹).

<i>Number of animals in each group</i>	<i>Minimum percentage incidence in experimental groups that can be regarded as significant ($P = 0.025$) *</i>
4	100.0
6	66.7
10	40.0
15	26.7
20	20.0
25	16.0
30	13.3
35	11.4
50	8.0
75	5.3
100	4.0

* Where no tumours appear in controls, and where equal numbers of control and experimental animals reach tumour-bearing age.

In most cases, however, tumours develop also in the control group. Table 1 gives the relative incidences necessary for a difference between the two groups to be significant for a P value of 0.05. This can be applied to each type of tumour separately or to the sum of all tumours observed in each group, if the tumour types are similar and therefore comparable. The establishment of negative results, particularly important in the investigation of food additives, presents more difficulty. Even with reasonable confidence limits it would be necessary to use several hundreds of animals per experimental group to exclude the chance occurrence of a 1% incidence of tumours above the incidence in the control group. It is unrealistic to suggest the use of such large numbers. Instead, it is necessary to rely on the fact that a response in a higher percentage of the animals is to be expected if dose levels are used far in excess of those recommended for human consumption.

Since a long induction period often precedes the appearance of tumours, consideration should be given to expected mortality so that a sufficient number of survivors remains for evaluation. In the case of negative response, at least 20 animals of each sex should survive for 2 years with rats and 80 weeks with mice in each of the different groups.

Diet

The basal diet used in any toxicological feeding study should be palatable and nutritionally complete for the species under investigation, permitting normal growth, reproduction and life span. However, excessive

¹ Boyland, E. (1957) *Acta Un. int. Cancr.* **13**, 271

TABLE 1. DIFFERENCES BETWEEN TWO GROUPS NECESSARY FOR SIGNIFICANCE AT A P VALUE OF 0.05 *

50 animals per group		40 animals per group		30 animals per group		20 animals per group		10 animals per group	
Number less affected**	Number more affected	Number less affected	Number more affected	Number less affected	Number more affected	Number less affected	Number more affected	Number less affected	Number more affected
0	6	0	6	0	6	0	6	0	5
2	8	2	8	1	8	1	7	1	7
5	13	4	11	3	11	2	9	2	8
10	19	8	17	6	14	4	11	3	9
15	25	12	21	9	17	6	13	4	10
20	30	16	25	12	20	8	15	5	10
25	35	20	29	15	23	10	17	6	—
30	40	24	33	18	26	12	18	7	—
35	44	28	36	21	28	14	20	8	—
40	47	32	38	24	30	16	—	9	—
45	—	36	—	27	—	18	—	—	—

* Derived from United States of America, National Research Council, Food Protection Committee (1959) Nat. Acad. Sci. Publ., 749. Note that this table applies only to each type of tumour separately.

** "Less affected" generally refers to control group.

amounts of any essential nutrient should be avoided. Full details of the composition of the basal diet should always be reported and in order to allow direct control over its uniformity, the diet should preferably be prepared in the investigator's own laboratory. When commercial rations are used, their composition with respect to both the ingredients and the nutrient levels should be known and satisfactory assurance should be obtained of their constancy of composition. In investigations for carcinogenicity it is essential to avoid contamination of diets and cages with insecticides, disinfectants, or detergents, and in the case of commercial pelleted diets, traces of lubricants.

To permit accurate recording of food consumption, diets should be in dry form, but if this is not possible owing to the moist or fluid nature of the test material, special precautions must be taken to ensure adequate measurement of intake. The effect of oxidative rancidity of fat components should be reduced by incorporating them at weekly or more frequent intervals, by storing under refrigeration fats, oils, and diets containing them, and by frequent replenishment of the feed cups.

Test materials should not be incorporated in diets if they are unstable or likely to react with any of their components. In such cases direct oral or intragastric administration may be employed provided the control animals are treated similarly with a placebo. When test substances are mixed into the ration, uniform distribution should be assured. If a solvent or vehicle is necessary it should be incorporated as well into the control diet. No solvent should be used if it leaves residue upon evaporation.

When test materials are incorporated into the ration of young, rapidly growing rats, cognizance must be taken of the diminution in food intake relative to body-weight. This also applies to female rats during pregnancy. In order to maintain uniformity of dosage during this period the concentration of test material in the diet may be adjusted as indicated in Table 2.

TABLE 2. EXAMPLE OF DIETARY ADJUSTMENT TO MAINTAIN UNIFORM DOSAGE LEVEL

Dose	Period after weaning at 3 weeks (weeks)	Food intake (g/kg day)	Test material (% of the diet)
100 mg/kg of body-weight	0- 2	120	0.084
	2- 4	100	0.100
	4- 6	75	0.133
	6- 8	65	0.154
	8-10	60	0.168
	10 +	50	0.200

Throughout a lifetime study, periodic records of food consumption, taken at appropriate intervals with representative animals from each test and control group, should provide the basis for checking the actual dosages of test substances consumed.

Examination of the animals

Regular and frequent determinations of body weight and food intake give a measure of the condition of the animals. It is of great value to have experienced personnel make frequent and regular clinical examinations of the animals. In this way cannibalism, for example, can be avoided. Moribund animals should be killed.

A complete post-mortem examination should be performed. The entire alimentary tract should be opened and organs often ignored (e.g., the pituitary) should be inspected whenever possible. Scientists trained in pathology and familiar with the diseases of laboratory animals, particularly tumours, should perform the autopsies or at least supervise these examinations. All organs showing macroscopic lesions should be examined histopathologically.

Tests by the oral route

Selection of dosage range

Doses should be selected carefully so that the maximal amount of information may be obtained. Tumour formation may be restricted to dosage levels which produce other chronic toxic effects or may be demonstrable at one or more lower levels. It is advisable, therefore, to select as many and as wide a range of doses as may be possible.

As a guide to selection of the dosage level of the agent under study, the investigator should consult available information upon its short-term (sub-acute) toxicity as described in the second report of this Committee. The highest dose used for determination of carcinogenicity should be one which produced a minimal to moderate amount of short-term toxicity. This may be described as the maximum dose for measurement of carcinogenic activity. If only one other dose is to be used, a level of $\frac{1}{4}$ to $\frac{1}{3}$ the maximum dose may be employed. If three doses are chosen, multiples of 1, $\frac{1}{3}$, and $\frac{1}{9}$ the maximum dose are suggested. It is important to aim at including a dose that does not materially decrease the life span.

From the data on acute oral toxicity, information upon short-term toxicity may be approximated.¹ It may also be possible to obtain satisfactory doses for measurement of carcinogenicity from the data on acute oral toxicity alone. One method² is to begin with daily doses of 5%, 10%

¹ United States of America, National Research Council, Food Protection Committee (1959) *Nat. Acad. Sci. Publ.*, 749

² Druckrey, H. (1957) *Arztl. Forsch.* 7, 449

and 20% of the oral LD₅₀. If one or more of these doses produces short-term toxicity reactions, then all three daily doses are halved or otherwise lowered; if no short-term toxicity reactions appear, then all doses are doubled or otherwise increased. By this method an approximate dose-response curve may be obtained.

Method of administration

Daily oral doses may be given in food, in drinking-water, or by intra-gastric intubation. Test materials are usually incorporated in the diet, and the concentration is adjusted so that the expected daily intake contains the dose required. The percentage to be added to food may be recalculated as indicated by changes in the daily consumption of food, which may be estimated at weekly or other intervals as suggested in Table 2 (see page 11). The dosage may be expressed as a concentration in the diet, in which case the food intake of the groups must be relatively uniform or known. An excess of this food mixture is given to permit the animals to eat *ad libitum*.

The daily dose may be given dissolved in drinking-water by a similar method of calculation. The solution should be changed daily and the water bottles kept thoroughly clean.

Giving the daily dose by gastric intubation permits more exact measurement of dosage. The dose should be dissolved, suspended, or emulsified and given in relation to body-weight.

Control groups

The incidence, time of appearance, number and type of tumour which may be found in the treated groups must be compared statistically with corresponding data in control animals. The control groups must be treated in a manner identical to that used in the dosage groups except that they are not exposed to the agent under study, neither must they be exposed to carcinogenic contaminants. The control group should be at least as large as the test group; if many test groups are involved, the size of the control group may be calculated as $\sqrt{N} \times$ number of animals per dosage group, when N equals the number of dosage groups (e.g., 100 controls for 4 dosage groups of 50 animals per dosage group). In special cases, positive control groups may be used which are given appropriate carcinogens for the purpose of comparison.

Tests by parenteral routes

Skin application

Test materials are applied in solvents, which should be non-irritant and free from carcinogenic contaminants; acetone is considered suitable for many materials. The concentration of the test material should be as high

as possible so that a small volume is applied. The back of the mouse and the ear of the rabbit are sites commonly used for this type of test. The material may be applied one to three times per week for a period of at least a year and the animals should be observed for their lifetime.

Subcutaneous injection

Only solvents which are non-irritant and not contaminated with carcinogens should be used, and control groups of animals must be injected with the solvent only. If an aqueous solution is injected the osmotic pressure and the pH should approximate to physiological values. For lipid-soluble materials, non-fluorescent tricaprylin or molecularly distilled triolein may be used. Injections should be given at weekly or monthly intervals for up to one year. The volume of each injection should be small to reduce the incidence of irrelevant effects. Insoluble materials can be injected as suspensions in suitable vehicles.

Intraperitoneal and intramuscular injection

Injection into the peritoneal cavity or into muscle might have advantages as compared with injection into subcutaneous tissue. These methods, however, need further investigation before they can be evaluated.

INTERPRETATION

The interpretation of the results of test procedures can be divided into two parts:

- (1) the consideration of the accuracy and significance of the experimental studies;
- (2) the evaluation of these facts, as well as epidemiological evidence, in terms of the carcinogenic risk in man.

When the risk has been estimated, appropriate action must be decided upon on the merits of each case.

Consideration of experimental studies

Design of experiment

The foregoing section of this report has indicated some of the main points that should be taken into account in the planning and execution of experimental studies designed to determine the possible carcinogenic risk associated with a particular chemical or agent. There are examples in the literature of attempts to interpret and evaluate unsatisfactory experimental work. It cannot be too strongly emphasized that the wide knowledge

and experience needed for these investigations are required from the start. No amount of subsequent pathological interpretation or statistical analysis will extract sound information from poorly planned or executed experiments. The Committee consider that first attention should be given to the quality of the work presented. They deprecate the publication of experimental studies of poor quality and interpretation which can only raise an ill-founded suspicion that a possible carcinogenic risk may exist. If such an investigation gives rise to a doubt about the safety of a substance that may occur in food, it is clearly in the public interest that further work be done. If, however, the evidence is not adequate to form a sound opinion, it would be wiser not to publish the information until adequate experimental evidence can be presented. Suggestions with regard to the information desired in publications are given in Annex 2 (see page 33).

Pathology

Since the assessment of tumour incidence is the crux of the evaluation of the carcinogenic risk, it is of great importance that all the animals should be subjected to adequate autopsy and histopathological examination. In many countries there appears to be a need for more people adequately trained in the pathology of laboratory animals.

Exact descriptions and precise diagnosis of tumours, as well as proper differentiation of neoplastic and non-neoplastic lesions, is not a unique need of investigations concerned with the possible carcinogenic hazard from food additives. As in other fields of medicine, clinical and experimental, it is imperative that the diagnoses be exact and clear. Otherwise, interpretation of food additive studies, and practical decisions based upon them, may be faulty.

Chemical substances or other agents continuously fed or introduced into the body by some other route, might result in a variety of toxic and/or neoplastic manifestations in tissues and organs—for example, vascular disturbance, degeneration, acute or chronic inflammation, hyperplasia, metaplasia, and benign and malignant neoplasms. These must be recognized, described, differentiated and classified. Vague and inexact descriptions and nomenclature such as "lesion", "pathologic change", "alteration", "tumour" or "cancer" are inadequate and confusing. More important, they may lead to unacceptable evaluation and dangerous or unfair practical decisions.

As an example, what are the various lesions that might possibly occur in the liver through the action of a chemical or agent incorporated into the diet? Some of those actually encountered in practice are various types of degeneration; dilatation of the sinusoids, in aggravated form appearing as blood cysts; acute inflammations; chronic inflammations and fibrosis (cirrhosis); cholangiofibrosis, sometimes mistaken for neoplasms; hyperplastic nodules of liver parenchyma (regeneration nodules), sometimes mistaken for benign

hepatomas ; and of course neoplasms such as benign hepatomas, cholangiocarcinomas and hepatocellular carcinomas. Obviously, precise recognition of pathological findings is only one of the factors in experimental design, execution and evaluation, but without it the others may lose importance and significance.

In the assessment of the carcinogenic risk it is not considered relevant whether the tumour is benign or malignant since the conversion of the first to the second must be regarded as possible.

Routes of administration

It is imperative that the carcinogenic risk of a food additive or contaminant be tested by the means or route involved in its actual use—that is, ingestion. The question arises as to whether other routes of administration of the chemical substance or agent—such as subcutaneous or intraperitoneal injection, or application to the skin—might provide additional information or proof of its potential carcinogenicity. Should this property be definitely established through feeding experiments, no need exists for investigations concerned with other routes of administration.

Suppose, however, that the results of adequate feeding studies reveal no carcinogenicity. It has been suggested that the safety of the agent should be further studied by injecting the material into the subcutaneous tissue of experimental animals, and this is practised by some investigators. In some instances, this procedure has resulted in the production of a high incidence of local sarcomas without inducing the formation of neoplasms in other tissues.

What is the biological significance of the results of such a series of experiments in relation to the problem of food additives? As the two-route studies have been described, it is obvious that the induction of local sarcomas is not a proof that the agent will be carcinogenic on feeding. However, such divergent findings are less satisfactory than those where the agent is non-carcinogenic by both routes—or by others that might be employed. Repetition and extension of the investigations might reveal evidence helpful in reaching a sound decision. The contradictory results of the feeding and injection studies might suggest the need for searching for a possible alternative substance which gives less reason for concern. For example, in some countries it is recommended that colours which produce local sarcomas when injected should be replaced by others, if functionally equivalent, which do not produce any significant tumour increase in animal experimentation by any route of administration.

Why does the induction of sarcomas, in the absence of carcinogenesis on feeding, leave uncertainty as to the value of the former findings? It is known that local sarcomas may be induced by chemical carcinogens, by some in minute amounts. However, certain plastics, metals and glass,

when placed subcutaneously in sheet or plate form, have also been demonstrated to result in sarcomas ; their activity appears to be related to physical form. The possibility exists that certain other chemical substances may induce sarcomas by this latter mechanism, yet reveal no activity in exhaustive ingestion studies. Nevertheless it has been ruled out that such substances may induce local sarcomas on the basis of their chemical reactivity. This is the status of our present knowledge, on which practical decisions must be made. Because of the absence of definite proof, decisions with regard to inclusion in permitted lists of substances giving rise only to local sarcomas at the injection site differ from country to country. This is unsatisfactory and clearly shows that in this area, as in others related to the food additive problem, more research is needed.

Dietary considerations

The genesis of neoplasms is influenced, in varying degrees, by the dietary and nutritional state of the host. Chronic caloric restriction strikingly inhibits the formation of many types of tumour. This effect has been demonstrated for all varieties of tumour investigated. Conversely, fat-enrichment of the diet augments the formation of certain tumours, but has no effect on others. As a generality, modifications of the dietary levels of protein, vitamins and minerals have lesser effects.

It is worth noting at least two important exceptions to this generality. A number of investigators have shown that high levels of dietary riboflavin retard the genesis of liver neoplasms in rats fed 4-dimethylaminoazobenzene, regardless of the general nature of the diet. On the other hand, some strains of rat having a high requirement for choline develop liver tumours when chronically fed a choline-deficient ration.

The inhibitory influence of caloric restriction and lower body-weight may have direct implications in investigations on food additives. Restriction of calories or deprivation of essential components of the diet may be a part of an experiment, but can occur also without the intention or knowledge of the investigator. For example, animals subjected to various agents (hormones, anti-metabolites, carcinogens, other chemicals, including the substance under investigation, or irradiation) may reduce their food intake or develop an increased need for particular dietary essentials. Intercurrent infections can cause diarrhoea or loss of appetite. These and other circumstances could bring on subnormal food consumption, altered metabolism, lower body-weight and a shortened life span. Should any of these conditions become a factor in an experiment, the genesis of expected tumours might be retarded—and possible induced neoplasms might not be evoked. Caloric restriction and low body-weight are not the common denominators of all inhibitory effects, but for more exact interpretation the nutritional status of the animals should be known.

Conversely, the addition to the diet of a lipid material or mixture, or of a substance dissolved in a lipid medium, may result in the potentiation or augmentation of carcinogenesis. If the experimental animal under study has a normal expectancy of a small incidence of one or more types of neoplasm, addition of lipids to the diet may result in a higher incidence, at an earlier average time of appearance. This cannot be interpreted as induction of tumours by a carcinogen.

It can be seen from this brief consideration of dietary factors that the general condition and nutritional state of the experimental animal, as well as the specific influence of a particular additive on its dietary intake and metabolism, may play a significant role in the production of neoplasms.

Action of carcinogens

It is known that various factors may be involved in carcinogenesis, including chemical agents, ionizing radiations, hormonal imbalances and viruses, but the mechanism is not completely understood. At the present time it is impossible, in many instances, to decide which or how many of these factors are concerned. For example, it may be difficult to decide whether a given chemical carcinogen is acting directly, through metabolites, through hormonal mechanisms, possibly by activating a virus or by some other mechanism. In the instance of certain food additives or contaminants reported to be carcinogenic (e.g., aminotriazole) it is thought that a hormonal mechanism is involved. In this case it has been suggested that such an agent should be called an "indirect carcinogen". No useful practical purpose appears to be gained by such a distinction at the present time.

It has been demonstrated, under certain experimental situations, that carcinogenesis may be a two-stage process involving an "initiator" and a "promoter" which may be separate agents. This aspect of carcinogenesis is so far insufficiently understood or generalised to be considered in the present practical context.

Evaluation of the risk to man

Extrapolation of animal experiments to man

Although it is agreed in principle that no assuredly safe level for carcinogens in human food can be determined from experimental studies at the present time, the Committee considers that it is advisable to deal rather more fully with this point. Research and development in the field of pharmacology and therapeutics is largely dependent upon successful extrapolation of animal experimental studies to man. It is apparent, however, that information about a pharmacologically active agent which rapidly produces some measurable effect of a reversible nature can be much more safely transferred from animals to man than that involving a process such as carcinogenesis

which is, so far as we know, irreversible and may take many years to become apparent. Furthermore, the therapeutic agent is normally administered intermittently and is subject to some measure of control so that unexpected deleterious effects are likely to be detected, whereas a carcinogenic substance in the food may be taken by people of all ages, perhaps daily throughout life, with little likelihood that the relationship to carcinogenesis occurring after a long period of time will be recognized. For these reasons it is considered advisable to reduce carcinogenic substances in the diet to the lowest practicable levels as stated in the general principles set out at the beginning of this report. From the scientific point of view, however, it may be said that carcinogens have been shown to have demonstrable dose-response relationships and that it is conceivable that dose levels exist that would not induce cancer. However, carcinogenesis is a complex process and this may vitiate such predictions. The uncertainty of the extrapolation of the safe dose to man, and lack of knowledge of the possible summing or potentiating effects of different carcinogens in the total human environment, preclude the establishment of a safe dose at the present time on grounds of prudence.

Human epidemiological studies

Chemical agents were first shown to be carcinogenic in epidemiological studies in man, which were undertaken on small occupational groups exposed to relatively large quantities of the materials for a long period. The establishment of a cause-effect relationship between most chemical agents and cancer is difficult unless the exposed group can be compared with control populations. Thus, it is possible to study the effects of dietary carcinogens when these affect circumscribed groups. If a food additive is consumed by the public, it may be extremely difficult to trace any effects it may produce. However, the matter can be investigated by the institution of epidemiological studies on groups concerned with the manufacture, or handling, or other occupational use of the food additive or contaminating substance, and this may be desirable in some cases. A number of substances that may be food contaminants are also used therapeutically, such as diethylstilboestrol, arsenites and thiourea; controlled studies in patients treated with relatively large amounts of these materials for long periods can be undertaken and may be important in answering many of the key questions concerning extrapolation of data from animals to man.

ACTION

It is the responsibility of toxicologists, oncologists and food scientists to evaluate the carcinogenic risk, but the final decision with regard to the action to be taken must depend upon a number of other considerations.

These scientists are not the only people needed to evaluate the significance of these other issues. The decision must be made by a group which should include, in addition, people with other qualifications and experience, such as agricultural scientists and public health authorities.

The basic issue involved is to balance risk against benefit from the point of view of the community and the individual consumer. Many matters such as the importance of the substance under consideration to the community as a whole, as in the case of DDT, or to food production, as in the case of a pesticide, or the natural occurrence of the deleterious agent concerned, as in the case of arsenic, must be given consideration.

So far as a carcinogenic substance is concerned, the action that may be taken is illustrated by the following. It is an accepted principle with food additives that they should be categorized in permitted lists, from which a carcinogenic agent can be excluded. With carcinogens that occur in the general environment and contaminate food, tolerance levels are set. In the case of secondary contaminants, such as carcinogenic substances extracted from plastics or waxes or resulting from contaminating processes, such as preservation by smoking, specifications are required which will ensure that the contaminating carcinogen is kept to the lowest practicable level. If a pesticide is found to be carcinogenic, residue may be avoided in food either by banning its use on food crops, by controlled use, or by insistence on the reduction of the residue to the lowest practicable level (that is, the level at which it is no longer detectable by a sufficiently sensitive method). Action along these lines would be compatible with the objectives set out in the general principles upon which this report is based.

RESEARCH NEEDS

In the course of discussions the Committee has noted many aspects of the problem that call for research, much more of which is required. In particular, the Committee wishes to emphasize that the provision of early and adequate support in the following fields might materially improve the safeguards that can be offered to the community with regard to the possible carcinogenic risk that may attend the use of food additives or the contamination of food.

(1) Methodological research in relation to testing procedures in animals :

- (a) investigation of the significance of results obtained using different routes of administration in various animal species, with special reference to the significance of tumour formation at the site of application ;
- (b) the validity and usefulness of combining feeding and parenteral administration in the same animal ;

- (c) epidemiological studies in animal colonies, aimed at improving the knowledge of the incidence of spontaneous tumours, the selection of the most appropriate species and strains for the problems under investigation, and the establishment and maintenance of healthy stocks of animals ;
 - (d) design and development of appropriate biometric methods for this field ;
 - (e) the study of time and dosage relationships in the investigation of responses to carcinogens of different potencies ;
 - (f) the development of reliable short-term biological tests for carcinogenesis.
- (2) Other research studies that might assist in the evaluation of the carcinogenic risk and for implementation of effective control measures :
- (a) development of better chemical and physical methods for the detection and analysis of carcinogens ;
 - (b) studies on antagonism, summation and potentiation, when more than one carcinogenic agent is involved ;
 - (c) epidemiological studies in man.

In putting forward these suggestions about possible areas for research, the Committee wishes to make it clear that it subscribes fully to the view that the first essential in research is the availability of people who are keen and competent to undertake it. While the fields indicated have emerged in the course of discussion as requiring attention, the list is by no means complete and, in the opinion of the Committee, the more people of good quality that elect to work in this or related fields, the better. The choice of one of the fields of work indicated here or adherence to any list of priorities is much less important than the recruitment of workers of high calibre. Research is dependent on people rather than places, projects, or priorities.

RECOMMENDATIONS TO FAO AND WHO

The Committee reviewed the past and present activities of FAO and WHO in the field of food additives and food contaminants. It recommends that the organizations should jointly :

- (1) continue to compile and publish specifications for the identification and purity of food additives and analytical methods relevant thereto ;
- (2) continue to collect and disseminate information with regard to food additive legislation in various countries ;
- (3) collect, compile, and distribute periodically data on the toxicology and carcinogenicity of food additives and food contaminants ;

(4) convene at suitable intervals the Joint FAO/WHO Expert Committee on Food Additives to evaluate the available information on the biological effects of food additives and food contaminants with a view to publishing lists indicating any major risks involved, including carcinogenicity, and the estimated safe level of total daily intake, for the guidance of those governments or governmental agencies who may wish to compile permitted lists ;

(5) arrange through suitable channels for maximal support for research in this field in appropriate laboratories in any of the member countries and for close integration between these laboratories and the two Organizations to facilitate exchange of ideas and information ;

(6) ensure the widest possible dissemination of information on their activities in this field.

SUMMARY

(1) The Committee based its considerations on certain general principles, and discussed in some detail the available evidence on the alleged carcinogenicity of a number of food additives and food contaminants and the action taken (see Annex 1, page 24), as a means of gaining a practical insight into actual problems that had been encountered. These general principles and the main points of interest that emerged from these discussions are included in the report.

(2) The Committee considered testing procedures for the evaluation of the carcinogenic risk that may arise from the use of food additives or from food contamination. It decided that the scope of the test required should depend on a number of factors, such as the nature of the substance, the extent to which it might be present in food, and the population consuming it.

(3) To enable a large number of substances, for which there is no indication of a carcinogenic risk, to be tested within a reasonable time, the Committee recommends the acceptance of a life-span feeding study in two species of animal (e.g., rats and mice) carried out along the lines recommended in the second report of the Joint FAO/WHO Expert Committee on Food Additives as a minimum safeguard.

(4) In special cases additional safeguards, such as the use of a suitable parenteral route of administration or studies in other species of animal, are recommended.

(5) A number of points relating to the identity and purity of the material under test, the animals to be employed and duration of experiment, numbers of animals and diet to be used, and examination of the animals are discussed. Some further information with regard to oral and parenteral tests is also given.

(6) In the discussion on interpretation, the Committee emphasizes the importance of good experimental design, execution and evaluation and full publication of results (see Annex 2, page 33).

(7) The Committee deprecates inadequate or premature publication in this field.

(8) The Committee strongly advises that pathological changes should be sought and accurately described, differentiated and classified.

(9) The Committee discussed difficulties in the interpretation of local sarcomas at the site of injection. It noted that action taken on this evidence alone differs from one country to another, and considers that this is unsatisfactory. The possible modifying effect of diet on tumour formation and the complexity of carcinogenic mechanisms were discussed.

(10) The Committee supports the view that any attempt to establish a safe dose for carcinogenic substances in the human diet at the present time would be unwise. Nevertheless, the Committee points out that permissible levels for some carcinogenic agents are unavoidable, as, for example, with naturally occurring substances or carcinogens of ubiquitous distribution.

(11) The Committee considers that epidemiological studies should be encouraged in appropriate fields.

(12) The Committee has reviewed the various control measures that may be taken, which are, of course, dependent on other factors in addition to the scientific evaluation of carcinogenic risk, and considers that they are compatible with the agreed objective of eliminating or reducing to a minimum the carcinogenic substances in the human diet.

(13) The Committee has considered research needs and makes recommendations.

(14) The Committee reviewed the past and present activities of FAO and WHO in the field of food additives and food contaminants and makes recommendations to the two Organizations for future action.

Annex 1**INFORMATION ON A NUMBER OF FOOD ADDITIVES
AND FOOD CONTAMINANTS
CONSIDERED BY THE COMMITTEE**

This Annex contains information considered by the Committee as part of the scientific background which deals with the available evidence on the potential carcinogenic risk, and action taken with regard to a number of food additives and contaminants during the past few years. It must be stressed that, in the time available to the Committee, it has not been possible to make this review as critical and exhaustive as might be desired and that the Committee has made no attempt to evaluate the evidence presented. The material only provides a means of obtaining a practical insight into some of the actual problems that have been encountered in this field. In this limited context, the Committee feels that the information is of sufficient interest to justify its inclusion as an annex to this report.

The substances, or groups of substances, discussed are :

- (1) Food colours
- (2) *para*-Phenethylurea (Dulcin)
- (3) Polyoxyethylene sorbitan monostearate (Tween 60)
- (4) Polyoxyethylene stearate (Myrj 45)
- (5) Carboxymethylcellulose
- (6) 4-Allyl-1,2-methylenedioxybenzene (safrole)
- (7) Tannic acids
- (8) α,α' -Diethylstilbenediol (diethylstilboestrol)
- (9) 2-(*para*-*tert.*-Butylphenoxy) isopropyl-2-chloroethyl sulfite (Aramite)
- (10) Thiourea and thioacetamide
- (11) 3-Amino-1H-1,2,4-triazole (aminotriazole)
- (12) 1,1,1-Trichloro-2,2-*bis* (*para*-chlorophenyl) ethane (DDT)
- (13) Isopropyl-N-phenyl carbamate (IPC)
- (14) Arsenical compounds
- (15) Seleniferous compounds
- (16) Substances that may be contaminated with carcinogenic aromatic compounds

(1) *Food colours*

The Joint FAO/WHO Expert Committee on Food Additives meeting in Rome in December, 1956, agreed that there are cases in which the use of food colours is justified and that the best method for regulation is the establishment of a list of permitted colours which have been tested adequately by animal experimentation. There is agreement that colours which produce cancer on administration by the oral route must be eliminated from these lists. There are, however, some colours which do not produce cancer in feeding tests but which on injection produce a significant number of sarcomas at the site of injection. In some countries it is considered that induction of such sarcomas is sufficient to indicate that a substance cannot be regarded as safe for man and in these countries it is considered prudent to reject such substances for use in food until more proof for safety is available.

The use of food colours which have not been tested sufficiently is undesirable—and particularly of those which are known to be carcinogenic (e.g., Auramine O, Tetramethyl diamino diphenyl cetonimine hydrochloride).²

As the components (e.g., β -naphthylamine in Yellow OB and AB) or other impurities present in colours may be carcinogenic, it is particularly important that rigid specifications, such as those listed in the fourth report of the Joint FAO/WHO Expert Committee on Food Additives be established and maintained for all food colours. In all cases the potential risks should be considered in relation to the advantages of their use.

(2) *para-Phenetylurea (Dulcin)*

Dulcin is a sweetening agent which has been used in the same manner as saccharin and cyclamates. It produced liver tumours in unstated incidence at 0.1% and above.¹⁰ This study has been repeated but no evidence of tumour formation in the liver was found.²⁶ Following the original observation, Dulcin was banned for use in many countries.

(3) *Polyoxyethylene sorbitan monostearate (Tween 60)*

Tween 60 is used as a dispersing agent in various foods and an anti-bloom agent in chocolate. It has been demonstrated to produce papillomas and a few carcinomas when applied to the skin of mice,⁴⁰ and sarcomas when injected subcutaneously in rats.²⁷ In addition, this material is an active "promoting agent" when applied to the skin of mice previously treated with a single sub-threshold dose of polycyclic aromatic hydrocarbon carcinogen.³⁸ It appears that there is no evidence that it is carcinogenic on feeding,³⁰ but definitely so by two other routes of administration.

(4) *Polyoxyethylene (8) stearate (Myrj 45)*

This material was proposed as an anti-staling agent for bread. The chronic feeding of Myrj 45 at a level of 25% in the diet to rats produced urinary bladder calculi in males, associated in some instances with bladder tumours, benign and malignant. Concentrations of 10% and less did not produce these effects.⁴³ Permission was not granted for the use of this material in the USA. The feeding of test materials at such high concentrations (25%) is reported to be most undesirable in toxicological testing.¹²

(5) *Carboxymethylcellulose*

This material is used as a thickening agent and stabilizer in ice cream and other food products in many countries. Feeding experiments for two years have been negative³⁹ but weekly subcutaneous injection in rats for a period of two years resulted in local sarcomas.²⁷

(6) *4-Allyl-1,2-methylenedioxybenzene (safrole)*

Safrole occurs to the extent of 75%-80% in oil of sassafras and to a small extent in other essential oils including cinnamon, nutmeg and mace. It is also produced synthetically. The characteristic flavour of certain soft drinks, notably root beer and sarsaparilla, is due principally to safrole. Despite their long history of use, recent unpublished studies of root beer concentrate and subsequently of safrole itself have disclosed its capacity to cause liver injury in rats and to produce tumours when fed at a level of 5000 p.p.m. (but not at lower levels). It should be noted that survival was affected at all levels except 100 p.p.m., only two of the group of 50 surviving two years at the carcinogenic dose level.

Long-term pharmacological studies in laboratories of the US Food and Drug Administration demonstrated safrole, which is the principal component of oil of sassafras, to be carcinogenic.⁴²

The use of safrole and of oil of sassafras was voluntarily discontinued in the USA by the beverage industry, and subsequently an official order was issued prohibiting the use of these products as well as iso-safrole and dihydro safrole in food. However, no action has been taken against the use of cinnamon, nutmeg or other natural substances containing low levels of safrole.

(7) *Tannic acids*

Tannic acids are used primarily as a clearing agent in the manufacture of some beverages. They also occur widely in foods. It should be recognized that this material is not a single chemical entity.

Hepatomas in rats have been induced by parenteral injection of some preparations, but not by feeding.^{24, 25} No action has been taken by any country against the use of this material in food. The case of tannic acids is another example of substances which induce tumours by parenteral administration but not through ingestion; in this instance, however, the tumours are remote from the site of injection.

(8) α, α' -Diethylstilbenediol (*diethylstilboestrol*)

The oestrogen diethylstilboestrol is used in the production of meat since it increases the efficiency of utilization of food. It has also been used for caponizing poultry. This substance has carcinogenic properties,⁹ but the amount added to the diet through these uses is small in relation to that already present from the oestrogens occurring naturally in various foods.

In some countries the use of diethylstilboestrol as a caponizing agent is prohibited, since measurable residues remain in edible portions of the fowl. It has not been prohibited in cattle-feed in the USA, although this is not true for some other countries.

(9) 2-(*para-tert.-Butylphenoxy*) isopropyl-2-chloroethyl sulfite (*aramite*)

Aramite is an acaricide formerly used on certain fruit crops at a tolerance level of 1 p.p.m. in the USA. The commercial product of aramite contains a small amount (5%-10%) of 2-(*para-tert.-butylphenoxy*) isopropyl sulfite.

The toxicological evaluation of aramite was based on 2-year feeding studies on rats and 1-year feeding studies on dogs, which were followed by carcinogenicity investigations in which three strains of rats, two strains of mice, and dogs were used.^{31, 32, 41}

As liver tumours were produced in two species the tolerance of 1 p.p.m. was revoked and a zero tolerance established. In the USA aramite had been used over a period of 8 years on food crops, but it is currently used only on non-food crops.

A sensitive analytical method is available for the determination of aramite.¹⁷

(10) *Thiourea and thioacetamide*

These two substances may be considered together since their action is similar. Both have been used as a fungicide on citrus fruits. The use of both was discontinued in most countries when it was demonstrated that feeding to rats produced hepatomas.⁹ These observations on thioacetamide were confirmed.¹⁸ Thiourea is well known to be thyrotoxic and it has also produced thyroid tumours in rats and mice.^{33, 34}

(11) *3-Amino-1H-1,2,4-triazole (aminotriazole)*

This substance is used as a weed-killer. It has been reported to cause thyroid adenomas and adenocarcinomas.²¹ A zero tolerance level has been established for this substance in the USA.

This agent is one of several goitrogenic substances that can give rise to adenomas and some carcinomas in the rat. Some of these agents, notably propylthiouracil, are widely used therapeutically, and there does not appear to be any evidence that they give rise to tumours in man.

(12) *1,1,1-Trichloro-2,2-bis (para-chlorophenyl) ethane (DDT)*

DDT is an important pesticide in world-wide use both as a space insecticide and directly on plant crops. It has also been applied to the bodies of men and animals as a de-lousing agent. DDT is present in food as a pesticide residue under official tolerance in many countries. It also occurs in food as a contaminant of milk and meat as a result of its presence as a residue in fodder and through its use as an insecticide in barns (not permitted in certain countries), warehouses, markets and domestic dwellings, etc.

The published data indicates that DDT induces a weak carcinogenic response in rats.⁸ Information on the effect of DDT on chronic feeding to species other than the rat, if available, should be reviewed.

Available information indicates that DDT has been subjected only to limited studies from the standpoint of potential carcinogenicity. More extensive work is urgently needed not only because of the ubiquitous distribution of DDT but because of its stability and tendency to concentrate in lipid depots.

Alternative insecticides, many in common use, do not appear to have been investigated with regard to their potential capacity to induce tumours.

(13) *Isopropyl-N-phenyl carbamate (IPC)*

IPC is a plant-growth regulator used as a potato-sprouting inhibitor and weed-killer in the production of vegetable crops. These uses result in the occurrence of residue in food. Feeding experiments were negative in mice and rats.^{6, 20} When administered orally to mice, concurrently treating the skin with a 5% solution of croton oil in olive oil, for one half-year, no tumours other than those of the skin were observed.⁷ The number of mice with skin papilloma and the total number of papillomas were slightly greater (borderline significance) than in control mice treated with a solution of croton oil alone. On the basis of the evidence available, a provisional tolerance was established in the Netherlands.

(14) *Arsenical compounds*

Inorganic arsenic : Potassium arsenite, principally in the form of Fowler's Solution, was used medicinally for many years before it was suspected as a skin carcinogen for man. By extension this effect has been attributed in frequently-quoted literature as being due to arsenic *per se*. However, it is well known that the toxicity of this element varies widely with the nature of the compound, and organic arsenicals, many of which have been used chemotherapeutically, have not been established to be carcinogenic.

The validity of certain epidemiological data on arsenic has been questioned.²³ A large collection of cases has been reported to be iatrogenic.²⁹ The occurrence of cancers of the liver and the skin of vineyard workers exposed to arsenical pesticides in Germany has been reported.^{35, 36} No experimental data have yet been published showing that any form of arsenic can give rise to tumours in animals.

One of the major problems arising from any consideration of arsenic and arsenicals is that this element is a common, naturally occurring, component of many foods, notably fish and shellfish. It will clearly never be possible to eliminate arsenic from the human diet.

Arsanilic acid and derivatives : Arsenic is used in many forms that may come into the diet in one way or another. Compounds of particular interest are arsanilic acid and certain of its derivatives which are used in poultry and pig feeds. Some of these compounds have been investigated by chronic feeding tests in rats but no data pertaining to the present problem are apparently available. Organic arsenicals might deposit some inorganic arsenic in the tissues but for this reason alone it cannot be concluded that the effect of organic arsenicals would be similar to that of Fowler's Solution. It would seem important that compounds containing arsenic be dealt with as individual chemical compounds and that the generic term "arsenic" should not be applied to all of them.

(15) *Seleniferous compounds*

Selenium is an essential nutrient for sheep and cattle and in certain parts of the world, notably the north-western states of the USA, there is a deficiency of selenium in natural fodder. This results in the development of a muscle disease which can be controlled by the addition of selenium compounds to the feed. Selenium compounds in the form of selenides and in seleniferous grain induced hepatomas in rats following long-term feeding.^{11, 28} Under the new food law in the USA, addition of selenium to animal fodder would be prohibited. It is permissible to transport selenium-containing fodder from other parts of the country where deficiency of selenium in soil is not present. This situation emphasizes the great importance of dosage levels since it seems clear that too little selenium is harmful

and gives rise to a deficiency state. However, too much selenium may cause toxic effects and may perhaps constitute a carcinogenic risk.

(16) *Substances that may be contaminated with carcinogenic aromatic hydrocarbons*

The materials considered are : (i) carbon blacks ; (ii) petroleum products such as mineral oils (liquid paraffins), paraffin wax, microcrystalline wax, petrolatum, lubricants, and solvents ; (iii) food prepared by smoking and other related procedures.

Carbon blacks and activated charcoals are used as food colours in certain countries and more extensively during food processing, especially for decolorisation. Mineral oil (liquid paraffin) can be used as a food additive or as an unintentional contaminant resulting from food technological processes in certain countries, and as a herbicide base with tar and pitch in the USSR. Paraffin, microcrystalline waxes and petrolatums are used largely in food packaging in different countries and to some extent as food additives (chewing gum, etc.). Smoking of food is used for purposes of preservation and flavour.

Certain carbon blacks have been shown to contain 3,4-benzpyrene as contaminants. However, it may be inactivated by adsorption. Petroleum products have been the subject of numerous investigations dating back to the investigations of the Tworts in the 1920 period. Several varieties of occupational hazard have been related to these products, including Wax Pressman's Cancer.¹⁹ Catalytically cracked petroleum residues have been shown to be highly carcinogenic to various species of laboratory animal, and the occupational cancer aspect has been discussed.⁵

No consistent evidence of the occurrence of overt carcinogenic action by any of the uncontaminated products mentioned has been published ; it is therefore felt reasonable to guard against the presence of carcinogenic impurities in all of them. Smoked food has been shown to contain 3,4-benzpyrene (1.9 to 10.5 microgram/kg in the case of smoked sausage, and 1.7 to 7.5 microgram/kg in the case of smoked fish).^{4, 13, 14} The carcinogenic hydrocarbon is found in the food as a result of the smoking process, and in much higher concentrations occasionally from contamination by soot.

The role of these compounds in cancer in man is emphasized by epidemiological studies in two groups :^{22, 44} a comparison has been made between Baltic fishermen and a population living inland under similar circumstances. It has been found that the fishermen eating considerable quantities of smoked fish have an incidence of all neoplasms three times that of the inland population who do not eat smoked fish, and four times that for gastro-intestinal cancer. A similar increase has been found in the workers engaged in the fish and meat-smoking industry. An increased incidence of gastric cancer in Iceland is considered to be related to the use of smoked foods.¹

In this instance the problem is one of contamination that has actually been demonstrated or that can be highly suspect as a possibility. The contamination may be a feature of certain of the processes used in manufacture.

Three different approaches have been taken to this problem. In the first instance a choice of alternative processes may be available ; thus in the instance of carbon black, the variety produced by the channel process is apparently not contaminated by 3,4-benzpyrene and has in certain countries been recommended as the best variety of this material for practical use, provided that it conforms to specifications of purity. In the instance of smoking of fish and meat, new processes are being designed to give the same practical results.^{15, 16} The use of uncontaminated wood and purified smoke extracts is also important.³⁷ Thus liquid treatment is being substituted both for preservation and for flavour. In the third instance, as with petroleum products, control measures may be instituted to analyse products and ensure that only those free of suspect materials or containing these at trace levels should be accepted. This should also apply to lubricants and packaging materials that may come into contact with food.

The problem raised in this section can be solved only by complete collaboration between food technologists and technologists of the various industries concerned. In the instance of petroleum products the problem is one of standardization ; the majority of available products are satisfactory and it appears probable that gross contamination is the exception. Standardization requires good analytical methods and it is hoped that work in this field will expand and finally yield universally acceptable methods than can be used for general control. It appears obvious that all these contaminants cannot be completely removed. There is equally no doubt that they can be drastically reduced, and experimental and epidemiological evidence suggests that this will have practical effects.

It may be noted that only the clearest examples of possible contamination with higher aromatic polycyclic hydrocarbons (of which 3,4-benzpyrene is but one example) have been mentioned. These materials have become ubiquitous and should be kept in mind as possible contaminants under various other circumstances.

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