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**ASSESSMENT OF THE  
CARCINOGENICITY AND  
MUTAGENICITY OF CHEMICALS**

**Report of a  
WHO Scientific Group**

WORLD HEALTH ORGANIZATION

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# CONTENTS

	Page
1. Introduction . . . . .	5
2. Mechanisms . . . . .	6
2.1 Mutagenesis . . . . .	6
2.2 Carcinogenesis . . . . .	7
3. Relationship of mutagenesis and carcinogenesis . . . . .	8
4. Comments on mutagenicity and carcinogenicity tests . . . . .	8
5. Threshold . . . . .	9
6. Assessment of hazards . . . . .	11
7. Conclusions . . . . .	12
8. Recommendations . . . . .	13
Annex. A proposed procedure for the assessment of health hazards of carcinogens at very low levels of exposure . . . . .	14

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CARCINOGENICITY AND MUTAGENICITY OF CHEMICALS

Geneva, 13-17 August 1973

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# ASSESSMENT OF THE CARCINOGENICITY AND MUTAGENICITY OF CHEMICALS

## Report of a WHO Scientific Group

A WHO Scientific Group on the Assessment of the Carcinogenicity and Mutagenicity of Chemicals met in Geneva from 13 to 17 August 1973. The meeting was opened by Dr P. Dorolle, Deputy Director-General, who welcomed the participants on behalf of the Director-General.

### 1. INTRODUCTION

For a number of years WHO and FAO have convened regular joint meetings of experts on food additives and on pesticide residues. The experts have been given the task of evaluating the toxicity and establishing acceptable daily intakes for food additives, pesticide residues, and contaminants that are important from the point of view of health and the supply of food.

In evaluating the toxicities of these chemicals, various problems have been encountered. One of these concerns the significance of exposure to very low levels of substances that have been shown to be carcinogenic or mutagenic in laboratory investigations. Some of these substances can be eliminated from the food; others cannot be readily eliminated. A realistic assessment must therefore be made of the health hazards, if any, that are involved in such exposures. This problem is not a new one, but recent developments in the relevant disciplines may be helpful in providing an answer.

In addition, the possible mutagenic action of food chemicals poses a definite health hazard. The WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives<sup>1</sup> reached the conclusion that while this problem cannot be ignored there are considerable difficulties in extrapolating from the experimental data to possible hazards of food additives in man. In recent years, however, a number of testing procedures have been developed. These involve the use of mammals, including human cell systems. A Scientific Group on the Evaluation of Testing of Drugs for Mutagenicity<sup>2</sup> was therefore convened by WHO in

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 348.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 482.

1971 to discuss, among other items, methods of testing and the interpretation of results. It is recognized, however that some new information has been accumulated in this field in the past two years.

Although the Group recognized that in many instances a critical analysis of benefits versus risks was valuable, discussion was limited to the evaluation of risks.

## 2. MECHANISMS

### 2.1 Mutagenesis

It is now accepted that the genetic material of all living organisms, with the exception of some viruses, is DNA. The genetic information is encoded in the sequence of base-pairs such that three bases specify one protein amino acid. The code is said to be universal, meaning that the same three bases (triplets) correspond to the same amino acid in all living systems. The double helical structured DNA proposed by Watson and Crick has made it possible to explain replication of the genetic material and mutations in chemical terms.<sup>1</sup>

A number of chemicals, including alkylating agents, analogues of DNA bases, and other types of molecule have been shown to induce mutations in biological systems, ranging from viruses to mammals. More recently the possible hazard to man from the presence of mutagenic chemicals in the environment has been recognized.

Mutations are classified as gene or point mutations, which may result from changes in one or a few bases, and as microscopically visible changes in the structure or number of chromosomes, which involve changes in many more bases. Point mutations may arise either by base substitution or frame-shift mechanisms. Base substitution can occur by incorporation of base analogues into the DNA, leading to mispairing on subsequent replication or by chemical reaction with a base already present in the DNA chain, giving rise to an abnormal base, which mispairs at the next replication. Frame-shift mutation involves addition or deletion of bases in the DNA and is induced by agents that, because of their size and shape, can become intercalated between the base pairs.

Heritable visible chromosome aberrations may follow exposure of cells to chemical mutagens. There are cellular mechanisms, described later in the report, that can repair lesions in DNA. The importance of DNA repair processes in human disease is illustrated by the rare condition xeroderma pigmentosum, which is genetically determined and in which

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<sup>1</sup> Watson, J. D. (1970) *Molecular biology of the gene*, 2nd ed., New York, Benjamin, p. 662.

cellular repair of DNA lesions induced by ultraviolet light is defective.<sup>1</sup> Sufferers from this disease have a greatly increased incidence of active skin tumours.

## 2.2 Carcinogenesis

Many, perhaps most, chemical carcinogens are thought not to be carcinogenic themselves but to require metabolic activation in the body to form active products which induce cancer. This activation is usually mediated by tissue enzymes, which occur mainly, but not exclusively, in the liver. Sometimes, however, activation is mediated by enzymes of the microbial flora of the intestinal tract. The terms precarcinogen, proximate carcinogen and ultimate carcinogen have been introduced by Miller & Miller<sup>2</sup> to describe respectively, the compound administered, its metabolites with increased carcinogenic potency, and the final metabolic product that is thought to react with a cellular component or components to induce the malignant transformation. In spite of the very varied chemical structures of the known precarcinogens there is evidence that many of them are converted in the body to electrophilic reactants, which interact with various nucleophilic centres in the cell, including nucleic acids, proteins, and protein-bound methionine. Similar conclusions apply to the metabolic activation of some mutagenic chemicals.

The need for metabolic activation of some carcinogens and mutagens has important implications for the design of *in vitro* tests for both activities. With some chemicals positive results can only be obtained in the presence of suitable metabolic activating systems.

Although the facts of interaction of the active forms of chemical carcinogens with cellular macromolecules are well established, the significance of these interactions for carcinogenesis is not yet understood. Reaction with DNA gives support for the idea that cancer can result from mutation of a somatic cell and thus for a close interrelation between carcinogenesis and mutagenesis. This is no more than a hypothesis, however, and various epigenetic mechanisms of cancer resulting from interaction with cellular RNA or proteins have been put forward. Recently, there has been much renewed interest in the idea that chemical carcinogens may activate latent tumour viruses already present in the cell. The above considerations probably apply only to those carcinogens that react covalently with cellular

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<sup>1</sup> Cleaver, J. E. (1968) *Nature (Lond.)* **218**, 562.

<sup>2</sup> Miller, E. C. & Miller, J. A. (1971) *The mutagenicity of chemical carcinogens: correlations, problems, and interpretations*. In: Hollaender, A., ed., *Chemical mutagens*, New York, Plenum, Vol. 1, pp. 83-119.

macromolecules. Other types of carcinogenesis that may involve indirect mechanisms are discussed later in this report.

### 3. RELATIONSHIP OF MUTAGENESIS AND CARCINOGENESIS

Current theories postulate similarities between the mechanisms of mutagenesis and the mode of action of major groups of chemical and physical carcinogens.

There is increasing evidence that many chemical carcinogens in their carcinogenically reactive form can induce mutations in microbial and some mammalian test systems. But it is impossible to assess whether or not these common properties of many chemical carcinogens and mutagens also point to common sequences of events resulting in a cancer cell or a mutated cell. Furthermore, some potent mutagens do not appear to be carcinogenic in any of the test systems used and certain carcinogens have not been demonstrated to be mutagenic. One major difficulty in the comparison of mutagenic and carcinogenic actions is the use of results obtained from different test systems. Induction of point mutations is reported mostly from studies in microbial systems, whereas chromosomal abnormalities have been observed in tissue culture and, more recently, *in vivo*. Carcinogenicity, on the other hand, is reported largely from *in vivo* studies in rodents. A second difficulty arises from the need for metabolic activation of many chemical mutagens and carcinogens. Until recently most *in vitro* systems used in mutagenesis bioassay have lacked this activation potential. It is thought that metabolic activation converting a precarcinogen into the "ultimate" carcinogen is analogous to the change from a premutagen to the ultimate mutagen.

### 4. COMMENTS ON MUTAGENICITY AND CARCINOGENICITY TESTS

The procedures for testing food additives and drugs and the interpretation of the results have been outlined in the fifth report of the Joint FAO/WHO Expert Committee on Food Additives<sup>1</sup> and the report of a WHO Scientific Group on the Principles for the Testing and Evaluation of Drugs for Carcinogenicity.<sup>2</sup> The *in vitro* test systems using cell transformation

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1961, No. 220; *FAO Nutrition Meetings Report Series*, 1961, No. 29.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1969, No. 426.

in tissue culture as an end point hold promise as a substitute test for the animal carcinogen bioassay.

Other test systems make use of the capacity of some, if not all, reactive forms of chemical carcinogens to interact with DNA. Mutagenicity tests may have value as a prescreening procedure for carcinogenicity. However, for the time being the development of a tumour, verifiable histologically, in the whole animal must be the ultimate test for carcinogenic activity.

Test systems for mutagenicity of chemicals have been evaluated by two WHO Scientific Groups.<sup>1, 2</sup> They agreed that no single test system can detect and characterize all mutagenic agents. Therefore, the use of several tests is desirable and these should primarily be done in mammals.<sup>2</sup> In addition a number of *in vitro* and submammalian test systems might be used to answer specific questions.

Many known mutagenic agents belong to classes of chemicals that need metabolic activation. Lack of metabolic activation has been one of the principal limitations of studies in *in vitro* and microbial systems. Furthermore, the activation process in submammalian systems, e.g., drosophila, might be different from that in mammals and man. The development of *in vitro* systems, including metabolic activation systems derived from mammals or man, may make possible rapid screening of substances. Data from such systems would be of value for setting priorities for more definitive mammalian testing.<sup>2</sup>

## 5. THRESHOLD

For most biological effects it is assumed from experience that a threshold and a no-effect level exist. Threshold dose levels in mutagenesis have been questioned on the basis of studies of radiation-induced mutations and because mutations may even result from a change in only one base pair in DNA. For carcinogenesis the existence of a threshold has also been questioned because of:

- (1) the self-replicating nature of the cancer cell.
- (2) the work of Druckrey and others, which has been interpreted to indicate summation of irreversible effects in carcinogenesis (this has been expressed by Druckrey in the equation  $Dt^n = k$ ,<sup>3</sup> where  $n$  is greater than 1).
- (3) evidence from experiments on tumour initiation and promotion in skin carcinogenesis indicating lasting change induced by one tumour-initiating event.

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 348.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 482.

<sup>3</sup>  $D$  = dose,  $t$  = time.

(4) the fact that cancer can occur in response to chemicals, even after single doses, long after their disappearance from the body.

(5) the possibility that cancer may result from mutation in a somatic cell.

The summation effect described by Druckrey and others is not questioned and his equation characterizing carcinogenic potency may be accepted. Nevertheless, every organism has a limited life span and in this sense there is, for each individual, a real threshold. Cigarette smoking is well known to cause human cancer in a dose-related fashion. The demonstration of a decline in the risk of developing lung cancer in ex-smokers means that these effects are partly reversible. Recent work on the initiation and promotion of tumours, in which application of the promoting agent was delayed for a longer time than in the earlier experiments, suggests that the effect of an initiating event may disappear, but this requires confirmation.

Knowledge of molecular biology has developed rapidly ; and it is now known that there are cellular mechanisms for the repair of DNA. These processes include single-strand and double-strand repair by excision or post-replication mechanisms. Most knowledge of DNA repair has come from investigations of microbial systems, but there are reasons to believe that similar processes occur in mammalian cells.

The repair of DNA usually shows exact fidelity but does not always lead to a perfect copy of the original DNA. Improper repair may result in the death or mutation of cells. Deleterious effects are more common after more severe DNA injury and when there is reduced capacity for repair. Impaired efficiency of repair may be genetically determined, e.g., in human subjects with the repair-deficient type of xeroderma pigmentosum. Several agents are known to interfere with DNA repair in microbial or mammalian cells *in vitro*.

In biological systems with an efficient DNA repair mechanism, the implication of an exposure threshold for point mutations and deletions is very strong. However, it is not established if such mechanisms are effectively present in various types of mammalian cell or if these mechanisms function *in vivo*. If cancer results from such mutations in a somatic cell the above conclusions regarding a threshold may apply to carcinogenesis.

A number of chemically induced tumours possess antigenic properties capable of inducing immunological tumour-associated rejection reactions.<sup>1</sup> The existence of immunological surveillance mechanisms that protect the

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<sup>1</sup> Baldwin, R. W., Glaves, D. & Pimm, M. V. (1971). In : Amos, B., ed., *Progress in immunology — First International Congress in Immunology*, New York & London, Academic Press, pp. 907-920.

host against neoplastic cells has been postulated.<sup>1</sup> This is supported by studies on host immunity to autochthonous tumours in man and others.<sup>2</sup> Immunodeficiency diseases lead to an increased risk of neoplastic disease.

The degree of importance of tumour-limiting responses remains to be analysed qualitatively and quantitatively. The dichotomy of the immune response — with mechanisms that both limit and facilitate neoplastic growth — should be kept in mind.

Further basic studies are needed before a correlation between chemical carcinogenesis and host immunity in man can be established.

From these considerations the existence of a threshold may be envisaged. Nevertheless the difficulties of determining a threshold for a population are great. Therefore, mathematically derived conclusions that it is impossible to demonstrate no-effect levels experimentally cannot be ignored.

## 6. ASSESSMENT OF HAZARDS

The term “carcinogen” has caused confusion because it applies to agents that are so varied in their quantitative and qualitative characteristics that their control requires many different approaches. However, common usage would seem to necessitate retention of the term. Chemical carcinogens can vary in potency in comparable test systems by a factor as high as 10<sup>7</sup>.

Since all chemical carcinogens pose a hazard, human exposure must be reduced to the feasible minimum. With compounds such as aflatoxin that may be active in microgram doses the achievement of this objective may raise formidable practical difficulties.

The action of the majority of carcinogenic compounds is associated with preliminary changes (e.g., hyperplasias, cirrhosis) the role of which is not clear. However there are some chemicals that give rise to neoplasms only after the induction of particular pathological effects. For example, the cancers of the urinary bladder observed in rats treated with Myrj 45 (polyoxyethylene monostearate) are thought to have been caused by the presence of bladder calculi induced by the chemical rather than by its direct action. A no-effect level for chemicals that produce tumours in this way may be established.

The evaluation of the carcinogenic effects of administered hormones must take into account their endogenous occurrence and participation in the regulation of physiological functions. If an intake of a hormone does

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<sup>1</sup> Burnet, F. M. (1964) *Brit. med. Bull.*, **20**, 154-158.

<sup>2</sup> Hellström, K. E. & Hellström, I. (1969) *Adv. Cancer Res.*, **12**, 167-223.

not increase its level beyond the physiological range, then it probably represents a no-effect level. The endocrine status of the test species used should be as close as possible to that of man.

The induction of cancer by some carcinogens is attributable to their physical characteristics. For example, some forms of asbestos are carcinogenic in man and animals. This appears to be related to the physical characteristics of the fibres.

In summary, therefore, it would seem logical that tumour induction be considered as a manifestation of toxicity to be studied as an individual problem in each instance. In some cases, the data available may permit the logical determination of a tolerance level whereas in others, currently the great majority, no such approach is possible.

From a practical standpoint, there is sometimes an irreducible environmental background level of certain cancer inducing compounds, such as aflatoxins and polycyclic aromatic hydrocarbons. The toxicologist must take this into account in his evaluation and recommendations.

## 7. CONCLUSIONS

(1) *In vitro* mutagenicity tests alone cannot yield definitive results applicable to man. Mammalian test systems are more promising but still require further development and experience.

(2) The relationship between carcinogenesis and mutagenesis requires further investigation. However, the association between mutagenicity and carcinogenicity of many compounds is sufficiently great to justify the use of mutagenicity tests as prescreening procedures for possible carcinogens.

(3) It is recognized that there are certain instances of cancer induction that may be secondary to an initial non-carcinogenic effect of a chemical.

(4) The role of modifying factors, enhancing or inhibiting the effect of carcinogens, must be considered.

(5) Assessment of risk must involve a knowledge of the environmental "background" levels of the chemicals concerned.

(6) Newer knowledge of DNA repair mechanisms and of immunological influences may have a bearing on the evaluation of the effects of low doses of chemical carcinogens.

(7) The possible existence of a threshold to the effects of both chemical carcinogens and mutagens should be envisaged (see section 5).

## 8. RECOMMENDATIONS

(1) WHO should promote the development of approaches and procedures for assessing the risks of low-level carcinogen exposure by extrapolation from experimental bioassay data.

(2) A knowledge of the environmental levels of carcinogens will be extremely useful in assessing their risks. Consequently WHO should promote more research into methods for the detection of these chemicals and coordinate and support international monitoring of the levels of certain of these chemicals.

(3) In those situations where carcinogens are unavoidable, or where the banning of a substance would impose a hardship or an unrealistic economic burden, the toxicologist must assess the risks associated with different levels of exposure. Proposed approaches for such evaluation include those made by Mantel & Bryan<sup>1</sup> and by Albert & Altshuler.<sup>2</sup> All the proposals suffer from lack of sufficient data to establish their validity and/or from arbitrary assumptions that lead to unrealistic estimates. Friedman (see Annex) has proposed the incorporation of the equivalent of a reference standard to make relative assessments possible. This whole area is of great practical importance and it is suggested that WHO should convene a separate meeting to evaluate this subject.

(4) WHO should encourage further work in the following areas:

(a) Basic research into mechanisms of carcinogenesis, including research into DNA repair, so that current empirical approaches may be replaced by one with a sound scientific basis.

(b) Studies on the effects of intake of compounds having hormone-like actions to elucidate the interrelationship of physiological and pathological effects that may have a bearing on the assessment of toxicity.

(c) Pathological examinations in studies of carcinogenesis where the mechanisms may involve possible secondary factors.

(d) Research into the design of a practicable test for point mutations in mammalian systems.

(e) Additional research concerned with the association of mutagenicity and carcinogenicity.

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<sup>1</sup> Mantel, N. & Bryan, W. R. (1961) "Safety" testing of carcinogenic agents. *J. nat. Cancer Inst.*, 27, 455-470.

<sup>2</sup> Albert R. E. & Altshuler, B. (1972) *Considerations relating to the formulation of limits for unavoidable population exposures to environmental carcinogens.* In: *Proceedings of the Twelfth Hanford Biology Symposium on Radionuclide Carcinogenesis.*

## Annex

# A PROPOSED PROCEDURE FOR THE ASSESSMENT OF HEALTH HAZARDS OF CARCINOGENS AT VERY LOW LEVELS OF EXPOSURE

by

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It may be envisaged that a threshold for carcinogenic activity exists. However, the threshold level cannot be accurately estimated at the present time, owing to the lack of relevant biological data. It should be remembered that any system of extrapolation from observations made at high exposure levels will give conservative estimates of the effects of low-level exposures if it ignores the existence of a threshold.

The first method of assessing the health hazards for carcinogens at very low levels of exposure was that proposed by Mantel & Bryan in 1961.<sup>1</sup> They took the position that the problem of determining what dose levels of an agent are safe, e.g., non-carcinogenic, cannot be resolved unless one first defines some level of permissible risk, no matter how small, rather than insisting on absolute safety. Furthermore, because of practical considerations and statistical variation, the determination of low-risk dose levels—for example, 1/100 million—cannot be made directly but only by extrapolation from observations at much higher levels. They describe a conservative approach for accomplishing this. In addition to an *arbitrary* definition of “virtual safety”, it is necessary to define an arbitrarily high statistical assurance level and a rule for extrapolation by use of an arbitrarily shallow slope. They defined “virtual safety” as a probability of carcinogenicity of less than 1/100 million at a statistical assurance level of 99% and a conservative probit slope of 1 probit per 10-fold dose increase.

The choice of 1/100 million is arbitrary. The choice of a 99 % statistical assurance level is, of course, also arbitrary, but it is obviously desirable for this level to be as high as possible. However, it may be that 90% would be adequate. The slope of a probit per 10-fold dose increase has been justified on the basis that the slope of the dose-response curve near zero is bound to be less than the slope in the range of actual observation. The choice of 1 probit rather than 1 logit per 10-fold dose increase cannot be

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<sup>1</sup> Mantel, N. & Bryan, W. R. (1961) “Safety” testing of carcinogenic agents, *J. nat. Cancer Inst.*, **27**, 455-470.

justified on the basis of available knowledge, but is an attempt to avoid being too conservative. Additional factors that are known to play a role in the determination of actual hazard are : (1) the probabilities that an individual will experience a given exposure ; (2) the likelihood that the risk being studied will be overshadowed by some other competing risk and therefore will not have become fully operative ; and, related to this, (3) the fact that the age of occurrence of cancer must also be considered when evaluating the hazard to health, especially as there is a long latent period for carcinogenesis, which usually gets longer as the exposure gets smaller.

Concern for this last factor is reflected in the approach to the assessment of health hazards of carcinogenesis at very low levels of exposure by Albert & Altshuler.<sup>1</sup> In their view, characterization of the carcinogenic response solely from the standpoint of incidence is not complete, since it ignores the age at which cancers occur. They point out that "the impact of cancer depends on the age of occurrence and is considerably less important in the very aged than in the young". In order to compare the dose-response relationships for cancer incidence with those for the age of occurrence of cancer and for the amount by which cancer shortens the life span, both in the exposed population as a whole and in the individuals who develop cancer, they developed a mathematical model. This is based on earlier work of Blum on the development of skin tumours in mice exposed to chronic ultraviolet irradiation, and on the work of Druckrey on a variety of chemical carcinogens and target tissues in rodents. They have extended the Druckrey formula and demonstrated its applicability to radiation cancer in mice exposed to radium-226 and to the tumorigenic response to cigarette smoking in man. The basic relationship is that  $Dt^n = k$  where "k" is a constant and "n" is always greater than 1, "D" is the average dose and "t" is the time that elapses before the occurrence of tumours.

The problems that Albert & Altshuler are concerned with at the present time relate to shape of the dose-response curve with respect to both time of cancer occurrence and incidence of tumours at very low doses, the variations in the value of "n" for different compounds, and the fact that at high values of "n", for practical purposes, the time of occurrence is independent of dose. Much more quantitative data on incidence and on the time of occurrence of tumours are needed for proper evaluation of the Albert-Altshuler approach to calculation of carcinogenic risks at low dose levels. Certainly, the experimental designs needed for assessing the health hazards

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<sup>1</sup> Albert, R. E. & Altshuler, B. (1972) *Considerations relating to the formulation of limits for unavoidable population exposures to environmental carcinogens*. In : *Proceedings of the Twelfth Hanford Biology Symposium on Radionuclide Carcinogenesis*.

of carcinogens at low levels of exposure are quite different from those needed to determine whether or not a substance is a carcinogen. Such designs must emphasize what has been, for practical purposes, ignored in the past, namely, a provision for determining with greater accuracy the time at which tumours appear, so as to have a quantitative tumour-time-dose relationship. Such experiments must make provision for interim animal sacrifices of groups of appropriate size for useful statistical evaluation. Such designs are now being developed in plans for work at the National Center for Toxicological Research.

Since the Mantel-Bryan approach does not take into account many of these other factors that determine actual hazard, the extrapolations derived by this system are obviously highly conservative. The calculated permissible levels obtained are between three and four orders of magnitude lower than those obtained by the more conventional approaches used to determine acceptable daily intakes. Since it seems evident that despite the theoretical and practical deficiencies of current safety evaluation procedures, they work very well for practical purposes, it must be concluded that something is lacking in the Mantel-Bryan approach that makes the extrapolation by this method unrealistic. Nevertheless, such estimates could be very useful if it is recognized that the choice of each of the three factors in their system is arbitrary and that, for practical purposes, other choices could be equally justified. For example, a probit slope of 1.5 rather than 1 would make a considerable difference in the extrapolation and still be consistent with the experience of those engaged in evaluating the safety of food components. A 90% confidence interval is not an unacceptable level of statistical assurance, and the meaning in real life of the probability of 1/100 million is hard to imagine in comparison with other everyday risks. Nevertheless, regardless of these specific details, the numbers obtained must be calibrated in some way to practical real life experience so that they can be set in the proper perspective for purposes of making benefit/risk comparisons that will lead to policy decisions.

Since the greatest need for such a system of estimating health hazards of carcinogens is for those substances that cannot be avoided in the food supply and in the environment generally (e.g., the polynuclear aromatic hydrocarbons), I would suggest that the first application of such a system as that of Mantel-Bryan must be to contaminants in conventional foods that are, without question, recognized as wholesome and safe. For example, it has been well demonstrated<sup>1</sup> that trace quantities of benzo[*a*]pyrene and other polycyclic hydrocarbons are present in a wide variety of foodstuffs.

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<sup>1</sup> Howard, J. W. & Fazio, T. (1969) A review of polycyclic aromatic hydrocarbons in foods, *J. Agric. Food Chem.*, 17, 527-531.

From published data<sup>1, 2</sup> and by applying factors to allow for the contribution of each class of foodstuffs to the total daily diet<sup>3</sup> it is possible to estimate the range of intake of benzo[a]pyrene or of all the carcinogenic hydrocarbons. For example, beefsteak containing 8 µg of benzo[a]pyrene per kilogram<sup>2</sup> and contributing 4.6% of the 1500-g total dietary intake of a 70-kg man would result in a daily intake of benzo[a]pyrene of 0.008 µg/kg.

Using a skin painting technique, Lee & O'Neill<sup>4</sup> have determined the dose-response relationship for tumour production by benzo[a]pyrene in skin. Berenblum & Haran<sup>5</sup> have estimated the potency of benzo[a]pyrene in producing carcinoma of the forestomach of the mouse. From these data, it is possible to calculate the probability of tumour incidence for any given intake of benzo[a]pyrene according to the procedures of Mantel & Bryan. The results of such calculations are given in the accompanying table. In addition to the results calculated for the probit slope of 1, as recommended by Mantel & Bryan, the table also shows values calculated for a probit slope of 1.5. The intakes of benzo[a]pyrene from 70 g of steak broiled over a charcoal fire would be 0.008 µg/kg per day. Using a probit slope of 1, the risks calculated from the skin tumour data are 2/10<sup>6</sup> and for the intubation data they are 8/10<sup>6</sup>. For a probit slope of 1.5, the tumour risks are, respectively, 1/10<sup>9</sup> and 1/10<sup>14</sup>.

The possible risks, according to the Mantel-Bryan calculation, depending on the actual content of benzo[a]pyrene in the total daily food supply,<sup>6</sup> can be seen in the table. It is, of course, necessary to have better data on the actual carcinogenic hydrocarbon content in order to make a proper estimate. On the assumption that the total carcinogenic hydrocarbon content is about 4 or 5 times the benzo[a]pyrene content, for an intake of 0.04 µg/kg per day the risk is 2/10<sup>5</sup> for the intubation data and 4/10<sup>5</sup> for the mouse skin data.

<sup>1</sup> Howard, J. W. & Fazio, T. (1969) A review of polycyclic aromatic hydrocarbons in foods, *J. Agric. Food Chem.*, **17**, 527-531.

<sup>2</sup> Lijinsky, W. & Shubik, P. (1964) Benzo[a]pyrene and other polynuclear hydrocarbons in charcoal-broiled meat, *Science*, **145**, 53-54.

<sup>3</sup> Association of Food and Drug Officials (AFDOUS) (1962) The annual per capita consumption of selected items of food in the United States, *Quarterly Bulletin*, **26**, No. 3.

<sup>4</sup> Lee, P. N. & O'Neill, J. A. (1971) The effect both of time and dose applied on tumour incidence rate in benzopyrene skin painting experiments, *Brit. J. Cancer Res.*, **25**, 759-770.

<sup>5</sup> Berenblum, J. & Haran, N. (1955) The influence of dose of carcinogen, emptiness of stomach and other factors on tumor induction in the forestomach of mouse, *Cancer Res.*, **15**, 504-509.

<sup>6</sup> This estimate depends on the completeness and quality of the data on carcinogenic polycyclic hydrocarbons in food and the assumptions regarding patterns of daily intake of various food products.

I suggest that the trace amounts of the carcinogenic polynuclear aromatic hydrocarbons do not constitute a health hazard, and that the risk calculated by Mantel & Bryan must be equivalent to our usual concept of safety. A numerical value calculated by such a procedure is, in fact, one way to define the meaning of "a practical certainty that no harm will occur from the use of this food".

I do not know, of course, whether these calculated probabilities of tumour occurrence are real risks, either for men or for mice. I doubt whether it will ever be possible to ascertain this with the desired certainty. Although these values are conservative, since they do not take into account important factors that should enter into any accurate calculation of hazard, I can see no problem in accepting a range of such values as a definition of safety. I believe that risk values calculated for contaminants in such ordinary food products as meat, potatoes, gravy, and apple pie provide calibration or reference points for the realities of safety in a system of calculation that is logical, but nevertheless, arbitrary.

As an initial step, I recommend that the Mantel-Bryan approach to assessment of risks from exposure to low levels of carcinogens be "calibrated" by calculation of the "Mantel-Bryan risks" for those carcinogens present as low-level contaminants in many foods that are generally regarded as safe and wholesome and have been widely used for many decades. Such calculations for the levels of polycyclic hydrocarbons, carcinogenic myco-

PROBABILITIES OF TUMOUR INCIDENCE CALCULATED ACCORDING TO MANTEL & BRYAN

Intake of benzo[a]pyrene µg/kg per man per day	Mouse intubation experiment <sup>a</sup>		Mouse skin painting experiment <sup>b</sup>	
	probit slope	probit slope	probit slope	probit slope
	1.0	1.5	1.0	1.5
0.0001	10 <sup>-11</sup>	10 <sup>-9.3</sup>	10 <sup>-10</sup>	10 <sup>-10</sup>
0.0005	10 <sup>-9</sup>	10 <sup>-21</sup>	3.3 × 10 <sup>-7</sup>	10 <sup>-15</sup>
0.001	6 × 10 <sup>-9</sup>	10 <sup>-19</sup>	2 × 10 <sup>-8</sup>	10 <sup>-13</sup>
0.002	3 × 10 <sup>-8</sup>	10 <sup>-17</sup>	10 <sup>-7</sup>	10 <sup>-12</sup>
0.004	2 × 10 <sup>-7</sup>	10 <sup>-16</sup>	4 × 10 <sup>-7</sup>	10 <sup>-10</sup>
0.006	6 × 10 <sup>-7</sup>	10 <sup>-15</sup>	10 <sup>-6</sup>	10 <sup>-9</sup>
0.008	8 × 10 <sup>-7</sup>	10 <sup>-14</sup>	2 × 10 <sup>-6</sup>	10 <sup>-9</sup>
0.01	10 <sup>-6</sup>	10 <sup>-14</sup>	3 × 10 <sup>-6</sup>	10 <sup>-9</sup>
0.02	10 <sup>-5</sup>	10 <sup>-12</sup>	10 <sup>-5</sup>	10 <sup>-8</sup>
0.04	2 × 10 <sup>-5</sup>	10 <sup>-11</sup>	4 × 10 <sup>-5</sup>	3 × 10 <sup>-7</sup>
0.08	7 × 10 <sup>-5</sup>	10 <sup>-9</sup>	10 <sup>-4</sup>	3 × 10 <sup>-6</sup>
0.10	10 <sup>-4</sup>	10 <sup>-9</sup>	2 × 10 <sup>-4</sup>	10 <sup>-4</sup>

<sup>a</sup> 30 animals  
<sup>b</sup> 300 animals

toxins, nitrosamines, goitrogens, naturally occurring estrogens, and ergot, which are usually found in low concentrations in traditional, safe foodstuffs, would provide “calibrations” for the “Mantel-Bryan risks”, that would render them useful for practical risk-benefit deliberations and practical decision making situations.

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