Potential Carcinogenicity of Food Additives and Contaminants

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Summary

The potential role in carcinogenesis of food additives and contaminants presents a complex problem in terms of assessing the risk to the general public. Long-term testing in laboratory animals is still the most feasible method for determining potential carcinogenicity of various chemicals. The disadvantages encountered in the present methods of animal testing are discussed and a review is made of the current status of particular food additives and contaminants under scrutiny as possible carcinogens. It is suggested that, since it may not be possible to remove all carcinogenic materials from the environment, methods to mitigate or neutralize their harmful effects should be sought. Greater cooperation is called for among food technologists, toxicologists, laboratory researchers, and epidemiologists in the decision-making process regarding the role of possibly carcinogenic additives and contaminants.

Introduction

Since the end of World War II, the potential role of food additives, both intentional and unintentional, as potential carcinogens has received considerable attention. The reasons for this arise primarily from our rapidly changing environment and technology, resulting in the introduction of many new agents in our daily lives, rather than from knowledge that such materials have, indeed, caused increasing incidences of particular cancers. We have known for many years that chemical compounds can induce cancer in animals. Toxicological testing has now been enlarged from its scope of pre-World War II days to include routine evaluation for carcinogenicity. Unlike other manifestations of toxicity encountered in routine tests, “carcinogenicity” has been selected for special treatment and the toxicologist in the United States has been told by the Congress how to use his findings. The Delaney Clause of the Food Additives legislation deprives the toxicologist of his usual prerogative to determine a rational approach to the control of a toxic manifestation of a substance. In addition the issue of carcinogenesis of “man-made” toxic materials has now become a “cause” and has a group of vociferous exponents, even among the oncologists, who have constituted themselves as “the” authority and readily reject any minimal deviation from this approach.

All this is very sad since, although caution is necessary in introducing new chemicals, particularly into our food supply, this should not require a gross departure from the general principles of toxicology, which, of necessity, use certain arbitrary guidelines. I mention this problem at the outset, since I believe it to be of central importance and likely to become more important as the effects of certain agents in humans are questioned.

Another key problem concerns the application of epidemiology in humans to inquiries into food additive carcinogenicity. The general assumption has been that, because food additives are so widely distributed and carcinogenicity takes so long to make itself apparent in humans, the investigation of such effects in population studies is all but impossible. That this is not the case has been demonstrated recently in studies undertaken into the potential carcinogenicity of saccharin. In this instance we are fortunate to have populations to compare statistically which have been very differentially exposed. Imaginative epidemiologists have constructed impressive studies using diabetic populations and a registry of bladder cancers created primarily to investigate occupational carcinogens. No doubt imaginative experimental design will be useful in the elucidation of the possible effects in humans of many other intentional and unintentional food additives. In diabetics there are other differences in diet that might be used. When the additive in question is a synthetic product or a manufactured product derived from natural sources, there will be occupationally exposed groups, exposed usually to many times the levels encountered in dietary use. An obvious example is DDT. In this case not only are the workers who are engaged in its manufacture exposed to high levels, but some agricultural users and crop dusters, for example, are (or were) exposed to high levels. In addition, it is known that human populations in different parts of the world have greatly varying levels of DDT in their fat. These statistics are available and have not been used adequately.

In the course of recent inquiries into the “significance” of fibers, possibly classifiable as “amphibole asbestos,” found in the water of Lake Superior, it became apparent that humans were exposed to vastly differing levels of various kinds of asbestos in many areas of the world, usually through drinking water. In many cases, this has obviously been true for years. These differences can be used to clarify problems touched on tangentially by animal experiments that are often so difficult to extrapolate.

I start with these examples, which may seem to some to represent a negative approach, in order to draw attention to a changing situation that I believe has been overlooked as a result of the different natures of the initial problems posed. Most toxicological evaluations of food additives are con-

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2 The abbreviations used are: DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenylethane); FDA, Food and Drug Administration; DES, diethylstilbestrol.
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cerned with new materials. Clearly, there is no way of determining the safety of such materials to humans except by using laboratory toxicology methods, particularly long-term feeding studies in animals. Such studies are not easy and I should like to discuss briefly some of the problems, particularly rapid test methods, types of carcinogens, and impurities.

Problems of Studying New Materials

Rapid Test Method. The most discussed rapid test methods are mutagenicity testing in bacterial systems, usually with mammalian liver microsomes added, and tissue culture. There is a degree of correlation between results obtained with mutagenicity tests and those obtained in whole animals. It is not a 1:1 correlation and is reported to vary from 65% to 75 or 80%. A carcinogen is not necessarily a mutagen in the whole system; nor is a mutagen necessarily a carcinogen. Tissue culture presents similar problems and has other disadvantages, particularly in limitations imposed by available cell types and ill-defined end points. Neither method can be used to classify a compound as a carcinogen potentially dangerous to humans. However, either might be used as a prescreen for a manufacturer who is trying to choose from a series of materials.

Types of Carcinogens. It is becoming increasingly more apparent that carcinogens are dissimilar and that this term is much less specific than many have believed. Hormonal carcinogens and those compounds that induce bladder cancer at very low incidence rates, following induction of bladder calculi, are 2 examples. The occurrence of hepatomas following administration of "enzyme inducers," such as phenobarbital, DDT, and dieldrin (1,2,3,4,10,10-hexamethoxy-6,7-epoxy-1,4,4a,5,6,7,8a-octahydro-exo-1,4-endof5,8-dimethanophthalene), is such an emotionally charged area that I would rather not discuss it in depth. Neither method can be used to classify a compound as a carcinogen potentially dangerous to humans. However, either might be used as a prescreen for a manufacturer who is trying to choose from a series of materials.

Impurities. The problem of impurities and interacting products is being reported more frequently. There are several categories involved. First, there are impurities that may occur as a result of the manufacturing process used; an example of this is o-toluensulfonamide in saccharin. Secondly, there are those compounds that react with a food constituent to give rise to a new compound of toxicological significance. Many examples of this result from interactions of secondary and tertiary amines and nitriles, or diethylpyrocarbonate and alcohol. A third situation concerns the metabolic conversion, either by the animal or by bacteria in the gut of the animal, to another, perhaps more toxic, compound, e.g., cyclamate → cyclohexylamine. There are a variety of similar instances familiar to the toxicologist but not necessarily concerned with carcinogenesis.

These examples illustrate the complexity of the problem facing the toxicologist. It is not enough to consider the compound in its own right. It is important that the toxicologist have the full cooperation of the food technologist. The mode of synthesis of potential impurities that arise from intermediaries or reactions is most important. Specifications of food additives are of immense significance. They are of course dealt with in the regulatory systems (WHO, FDA, Food and Agricultural Organization, etc.), but there is often a hiatus where the toxicologist does not look into this matter in depth. More frequent contacts between those involved are desirable. A thorough knowledge of the metabolism of the test material is a prerequisite for the design of a maximally effective test.

Additives

I should now like to discuss some of the additives, intentional and unintentional, that have been found in one way or another to be carcinogenic and delineate some of their salient features.

Fluorenlylacetamide. This proposed pesticide (also known as 2-acetylaminofluorene) represents a landmark in this field. It was tested for chronic toxicity by Wilson et al. (20) in 1941. It was found to have a wide range of carcinogenicity inducing a series of different malignant tumors in a variety of organs of the rat. Since that time it has been found to have a wide range of activity in a large number of species, and much is known about the preliminary steps in its metabolism. It was never used as a pesticide and I believe that it would be difficult to find anyone who would doubt the wisdom of the decision not to market this compound.

Aramite. Aramite [2(3-tert-butylphenoxyl)isopropyl-2-chloroethyl sulfite] is the classic example of some of the difficulties in this field. The compound was tested and found to produce hepatomas in rats (12). A committee of the National Academy of Sciences was assembled and spent much time debating whether the hepatomas were regenerative nodules or benign or malignant neoplasms. They did not arrive at a consensus and decided that the company in question should retest Aramite. Aramite was retested at considerable expense and produced the same lesions in rats and cholangiocarcinomas in dogs. It was accordingly decided, perhaps because dogs were man's best friend, that Aramite was undoubtedly dangerous, and it was thereupon banned.

Thiourea and Thioacetamide. These were at one time used as fungicides. They produced both hepatomas and thyroid tumors in studies undertaken by the FDA, and both were subsequently removed from the market. Thiourea and associated compounds have, of course, been administered to humans for years in the treatment of thyroid disease. It would be rather nice to know more about this. Naturally, there is evidence available from the patients and, in general, the compound would appear to be noncarcinogenic to humans. I would have thought, however, that perhaps this was a field for additional exploration by our colleagues in epidemiology.

Aminotriazole. This was an offending substance, similar to thiourea, also inducing thyroid lesions. There was a great
argument, still unsettled, about whether or not some of these were malignant. Aminotriazole (3-amino-1H-1,2,4-triazole) was the substance that caused removal of the cranberries from the United States market over one sad Thanksgiving some years ago, and it has been unavailable ever since.

**Isopropyl-N-phenylcarbamate.** This is an anti-sprouting agent found to be an initiator of carcinogenesis when given to animals whose skin was subsequently painted with croton oil (16). This particular experiment, which was rather limited, was taken very seriously by our friends in The Netherlands and somehow or other it was banned there, but nowhere else.

**Maleic Hydrazide.** This compound, when injected into newborn mice, produced tumors (3). However, such injections must contain adequate amounts of hydrazine, which occurs as a reaction product in maleic hydrazide, and it is impossible to obtain maleic hydrazide free from hydrazine. Maleic hydrazide was used widely in agriculture by the tobacco industry. It was and is a difficult compound to regulate.

**Chlorinated Hydrocarbons**

DDT, Aldrin, and Dieldrin. All of these produce hepatomas in the mouse (15, 18). Studies in other species are rather hazy. The rat studies with some of these compounds would appear to be equivocal. There is a negative study with DDT in the hamster; as a matter of fact, DDT is nontoxic in the hamster. We are unable to get enough DDT into the hamster even to cause a detectable effect. DDT, I personally happen to think, is one of the most useful compounds ever invented by mankind, perhaps second, or even parallel to, the antibiotics in lifesaving. This question of hepatoma induction in the mouse is very tricky and is currently charged with emotion. I do not want to discuss it in depth, but I mention the subject since some of you may not be aware of the occurrence of hepatomas with aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,8a-hexahydro-exo-1,4-end-5,8-dimethanonaphthalene), dieldrin, and DDT and, more recently, with the solvent trichloroethylene, chloroform, and with some other chlorinated hydrocarbons used in food. Carbon tetrachloride (1, 2), which most people think of as being used in the cleaning industry, occurs as a low-level residue in bread, and chloroform occurs as an additive in toothpaste and cough mixtures. All these compounds produce hepatomas, as does phenobarbital. These findings constitute an issue that must be resolved. We must discover what this really means; possibly, there is an association between enzyme induction and the occurrence of these tumors.

**Vinyl Chloride.** This chlorinated hydrocarbon appears to be in a different biological class from those discussed thus far. It would be most interesting to know how different it is and more about mechanisms of the entire group. This compound introduces a series of general problems for the practical toxicologist. In an initial rat study vinyl chloride was shown to induce tumors (17); the study was not taken as seriously as was warranted, including, I must confess, by myself. With hindsight the importance of the study is apparent. The next occurrence was the overt demonstration of the carcinogenicity of vinyl chloride in humans; it is known to cause hemangiosarcomas (rare tumors indeed and rapidly fatal ones) in men apparently receiving relatively large exposures. Since that time the compound has been shown to be similarly carcinogenic in several animal species. I do not believe that control of the industrial hazard poses a great problem to the regulators. The widespread use of the material in food packaging, on the other hand, requires extrapolations that can only be made arbitrarily. In some animal systems the dose required to induce neoplasms is low, and surely this may be true for humans. It would seem reasonable to be as prudent as possible.

A major question now will be the relationship of these findings to the predictability of hazard from related compounds that find their way into the environment. A recent finding in the bioassay program of the National Cancer Institute concerned the demonstration that trichloroethylene induced hepatomas in mice. The biological result thus far reported suggests similarities to DDT rather than to vinyl chloride; however, further testing might change the picture. The compound is widely used for dry cleaning and as an industrial solvent and can be a residue in foods. The regulatory problem here is quite difficult, especially since banning this compound may easily result in the substitution of related, untested compounds.

**Nonnutritive Sweeteners**

Three compounds in this category, for some obscure reason, have absorbed as much funding for testing as was once the budget of the National Cancer Institute for a whole year. Test after test has been performed with these particular agents.

**Dulcin (p-Phenetylurea).** This sweetener was demonstrated to induce hepatomas in a test (4) that was contradicted in a subsequent investigation (8). In this instance the material was also toxic in other ways and no further energy was expended upon it. It was not deemed suitable for use following the initial report of carcinogenicity, and no practical use was made of the subsequent negative studies.

**Saccharin and Cyclamates.** There has been a succession of experiments demonstrating that saccharin and cyclamates may indeed be carcinogenic. Epidemiologically, saccharin would seem to be given a clean bill of health, although all the data have not yet been published. Cyclamates have managed to become one of the curiosities of toxicology. Somehow over a period of years the usual mechanisms for regulation of these materials have been by-passed, and decisions have been made by a series of people who do not usually take part in this aspect of control.

There is not the slightest doubt that compounds such as cyclamate require extensive testing and that an error was made originally when cyclamate was allowed on the market in the United States without having been tested properly. Cyclamate was classified as a "generally recognized as safe" material before it had been tested chronically in an animal system. I think a compound that might be ingested in as high a dose level as 3.5 g/day without doubt required
thorough testing. If overt carcinogenicity had been discovered there would be no problem. It is when borderline results occur repeatedly that serious problems arise. For the toxicologist, the saccharin-cyclamate issue is rather difficult because it raises, among other questions, the problem of how extensive these tests really should be. Saccharin has been found to produce tumors when given as 7.5% of the diet through 2 generations of the animals. Should one really do a test like that? What are the implications? Should we ask people to test additives in 2 generations from now on? These and similar complicated questions face the toxicologist every day.

Food Colors

For a variety of reasons the food colors have received special attention over the years and have, at times, played a central role in the determination of basic principles for the elucidation of control measures for food additives. Some few years ago it was the fashion to test these substances by s.c. injection; several food colors resulted in the induction of s.c. sarcomas. After much acrimonious discussion it was extensively additional tests. However, the general principle with the caveat that the induction of a sarcoma by a very small quantity of a test substance does, of course, indicate an adverse effect requiring the toxicologist to carry out extensive additional tests. However, the general principle that food additives should be tested p.o. is obviously sound.

One of the 1st carcinogens to be used extensively in the laboratory was p-dimethylaminoazobenzene known previously as a food color, butter yellow. This compound is known primarily for its ability to induce hepatomas in the rat, appears to be relatively limited in its carcinogenicity to the liver, and does not manifest equal activity in other species tested. The presence of residues of the well-known carcinogen 2-naphthylamine in Yellow OB and Yellow AB resulted in control of these compounds. Red 2 still remains controversial not only as a possible carcinogen but as a teratogen. In this instance the problem of specifications plays a central role.

Auramine is a compound whose manufacture causes cancer in man which seems to result from an as yet unidentified impurity.

Additional Compounds

Next I shall mention a series of additional “problem children” to illustrate the complexity and confusion that may exist. The 1st compound, polyoxyethylene sorbitan monostearate or Tween 60 (14), is a well-known emulsifying agent used in numerous products and it is a promoting agent. If you paint an animal on the skin once with a polycyclic hydrocarbon, you can follow this up by swamping the animal for the rest of its life with Tween 60; as a matter of fact, the effective dose is approximately the weight of the animal. A mouse must be painted with 20 to 30 g of Tween 60 to produce skin papillomas and most of those regress.

Nevertheless, notwithstanding the exact nature of these experiments, serious consideration was given to the removal of Tween 60 as a food additive. The compound has been fed and does not appear to produce tumors on its own, but there is the suggestion that it allows carcinogens to penetrate more quickly. Those experiments are in the literature, but I think are not too convincing that Tween 60 constitutes a hazard.

Polyoxyethylene stearate (Myrj 45) (5) is a compound that was used in a manner that I consider somewhat undesirable. It was marketed as a product called Sta-Fresh and was added to bread to make it feel fresh. Myrj-45, when fed to rats as one-fourth of their diet, resulted in the formation of bladder calculi and subsequently a certain number of papillomas. The results were sent to the FDA. The FDA assembled a very judicious committee, some of whom decided that this was an inappropriate test, and Myrj-45 was allowed to remain on the market at a very low level.

DES, of all carcinogens, may illustrate the complexity of the issues best of all. In recent years it has been demonstrated that some of the daughters of women who received rather large doses of DES (6) to prevent threatened abortion are developing carcinomas of the vagina. This is a terrible and terrifying occurrence. There has been considerable concern, perhaps most of all in the Congress, with the use of DES as a food additive for cattle. I am unable to discuss the economics of this situation in depth even though I come from a cattle-producing area. However, I must confess that I have been unable to understand the logic that results in the elimination of this use of DES on the basis of the presence of ppb residues in beef liver when new therapeutic uses of the drug such as the “morning-after pill” are approved, when extensive postmenopausal use of the drug is hardly discussed, and when there is no great concern expressed about the indiscriminate use of more potent estrogens in “the pill” although these agents produce similar effects in animal test systems. There is no great emphasis as far as I can tell applied to occupational hazards from manufacturing these materials even though gynecomastia has been reported.

There is a degree of illogic about all this that should be considered since the matter arises in other situations, perhaps in a less obvious manner. I cannot, obviously, discuss this matter without stating my own position. I believe that the ppb residues of DES in beef liver are most unlikely to pose a hazard to humans and that if they do it would be small. This is a situation in which a benefit-risk analysis must be brought to bear upon the regulatory decision. Insofar as the postmenopausal use of DES is concerned, it would seem again that the medical profession should collect and collate data that will demonstrate the usefulness and possible hazards in a proper manner. In view of recent warnings that “the pill” is associated with an increase in fatal coronary artery thromboses in women over the age of 40, such a study should have a wide scope. I would have no doubt that the morning-after pill should not be used in view of the data on carcinoma of the vagina in young women whose mothers received this drug in pregnancy and in view of the availability of other methods for inducing abortion. A major problem is the proper evaluation of the
effects of various versions of “the pill” which, it is now agreed, induces hepatomas not only in rodents but apparently in a small proportion of women. The potential hazard from estrogens administered in these doses to such large populations cannot be overemphasized. It is astonishing that we could have become so fragmented in our decision making; let us hope that it is not too late to rectify the matter.

Safrole was found originally to produce hepatomas in rats (9) and illustrates the danger of not looking into things in detail in several species, since it was subsequently found to produce tumors of the esophagus as well (10). I think that we are well off without safrole.

Korpássy (7) showed that tannic acid could induce liver tumors in rodents. The extrapolation of this result to humans is difficult. There have been suggestions of an association between hot tea drinking in Iran and cancer of the esophagus; but these have not been substantiated. Dr. Bogovski, formerly of International Agency for Research on Cancer, has been undertaking experiments with tea in rats, but I have not seen the results. In view of the complexity of the chemistry of tannic acids, much additional work in this field would be needed before practical use could be made of this information.

Asbestos is used widely in the food industry. It is used for filtering processes in making beer and for a variety of other processes in producing alcoholic beverages. As I understand it, one of the highest asbestos contents is found in Italian vermouth. Again, I think that there are many ways of looking at these problems epidemiologically and I would concur with views expressed earlier in this session that the data on the effects of ingested asbestos are, at best, only suggestive.

The nitrosamines have been discussed in depth at this conference, and I think there is little more to be said about them.

The findings that we were lucky enough to encounter with inhibition of nitrosamines by vitamin C (11) have some general implications. The potential of being able to neutralize carcinogenic food additives or inhibit their formation, I think, is an approach that must be looked into in detail. There is no question in my mind that situations exist that indicate that it will not be possible to eliminate all carcinogens. Accordingly, other ways must be found to deal with this situation. The use of vitamin C appears to be one means of preventing formation of nitrosamines from nitrites and seems, to some extent, to be practical.

The presence of these very low levels of carcinogenic nitrosamines we now see (nitrosopyrrolidine in cooked bacon, for example) has, I think, been dealt with very wisely. The FDA, in such instances, has done nothing drastic concerning these compounds, and I think that this is a reasonable approach. Were the quantities larger, on the other hand, I would then say that action should be taken. In the end, it is essential that we behave like reasonable toxicologists. Each one of these problems must be analyzed in its own context and own right, and then a logical and reasonable approach should be taken.

Last, I would like to mention the question of polycyclic aromatic hydrocarbons in smoked foods. Dr. Weissberger, in an earlier address (19), alluded to this matter very briefly. Cancer associated with smoked fish in the Baltic States is often discussed and the Icelandic findings are similar. In this instance, however, there are means of eradicating selected carcinogens from some of these smoked foods. For example, by using liquid and condensed smoke, it is possible to produce foods that taste virtually the same. The liquid smokes are made from wood smoke, condensed, and used in flavoring agents. In producing a substitute, the heating and preserving processes would have to be replaced in some other way, but by using the substitute most of the polycyclic hydrocarbons could be removed. There are acceptable daily intakes set for foods such as these, as well as some standard procedures.

The essence of the story is that, if you can smoke a food without producing these polycyclic aromatic hydrocarbons and gain the same result, you might as well get rid of them. There are, however, numerous other materials in these smoking fluids that the toxicologists haven’t looked at. These factors are enormously complicated and they direct one to the fact that, despite all the attention given food additives, a minimal amount is being paid to cooking procedures, of which smoking is one. The vast number of things that may occur in cooking constitute a Pandora’s box just waiting to be opened by some fortunate young scientist who is going to make a name for himself in the future.

There are clearly thousands of compounds that have not yet been tested. I think I can safely predict that in the next year or two items such as flavoring compounds, of which there are hundreds, will be found to be mutagenic by the droves. We won’t know what to make of it and it will be a very difficult problem indeed.

The next speaker, Dr. Schwartz (13), will deal with 2 elements of interest to me. One of these is the matter of arsenic, which is a situation where everything is reversed, in that there are epidemiological data but no animal data whatsoever. What we make of that, I don’t know. Conceivably, there is something else associated with the arsenic that has been looked at by the epidemiologist. It is, however, a problem worthy of our attention and one that should be sorted out.

The other matter concerns the excessive addition of iron to our diet, which deserves considerable attention, in view of the facts that hemosiderosis is undoubtedly associated with an increased cancer risk in humans and that there are a variety of iron derivatives that have been shown to produce tumors in animals under certain circumstances. The association of iron as a carrier in the lungs is another important consideration in this light. Currently, there is somewhat of a preoccupation with adding iron to numerous items including, as I discovered recently, black olives to make them shiny black. In California, ferrous glutonate is a food additive used under these circumstances.

In conclusion, let me say that in spite of the harsh words I may have had to say about the Delaney Clause and the philosophy surrounding it, much good has come as a result. At the time of the enactment of this legislation, we were lax indeed in our attitude towards compounds found to be carcinogenic in the laboratory. Some years ago I subscribed...
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to the philosophy that all materials found to be carcinogenic in the laboratory should be totally eliminated. Since that time much research has been undertaken and many new facts have emerged making it clear that "carcinogens" encompass a wide range of materials dissimilar in their dose requirements, in their mode of action, and in their potential danger. They are not only man made but also occur as a result of naturally formed toxins. The practical nature of the problem requires a broad approach that cannot be as limited as seemed possible 2 decades ago. We must beware lest our rigidity born of justified anxiety makes us seem to be extremists.

Finally, it has been a great privilege to have been present at a meeting largely devoted to epidemiology; cooperation between the epidemiologist and the laboratory toxicologist is of major importance and will, I believe, eventually solve most of the problems I have discussed today.

References

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