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THE EVALUATION OF COSMETICS AND TOILETRIES FOR CARCINOGENICITY

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SYNOPSIS

Evaluation needs to be based on an up-to-date concept of carcinogenesis. Cancer is a group of diseases each with multiple contributory causes. Genetic, viral, physical and chemical factors in various combinations influence the risk of development of particular kinds of cancer. Calorie intake, hormonal status and naturally-occurring chemicals can be as important as man-made chemicals as determinants of cancer incidence. The risk of development of most kinds of cancer increases logarithmically with age. In old age 25% of humans develop one or more cancers. In elderly laboratory animals incidences of up to 100% are frequently encountered. Cancers develop by a multistage process. The first stage is mutation-like and tests for mutagenicity are relevant to the detection of agents (tumour-initiators) which cause this first stage. Subsequent stages may also be mutation-like but alternative mechanisms have been demonstrated (e.g. immune-suppression, irritation). In the intact animal, cells that might give rise to cancers tend to be carefully protected from contact with highly reactive carcinogens. Thus substances absorbed by the gut tend to be detoxicated in the liver, stem cells for replenishing the gut mucosa lie deep in the crypts and not on the tips of villae, DNA damage is often reparable etc. For these and other reasons the demonstration of mutagenicity is not tantamount to the demonstration of carcinogenicity. Nor does the malignant transformation of cells in culture prove that a substance is capable of causing cancer in the intact animal. It is presently generally accepted that long-term animal tests are needed for the demonstration of carcinogenicity and that negative data from such tests are required for the demonstration of lack of carcinogenicity. There are, however, many pitfalls in the design, execution and interpretation of long-term animal tests and these need to be very carefully taken into account.

The evaluation of Cosmetics and Toiletries for carcinogenicity usefully begins with a consideration of chemical composition, chemical structures and the possible presence of minor contaminants. It is important to develop tight specifications for materials that are to be tested biologically and essential that tests are carried out on the materials of similar quality to those proposed for human use. Metabolism and target organ studies in several species may help one to choose an appropriate species for use in a carcinogenicity test. The route of administration should normally be the same as that for man, unless to match human tissue levels another route seems more appropriate. Several dose levels ranging down to those proposed for human use should be studied. It is debatable whether the effects of maximum tolerated doses should be investigated when these vastly exceed those to which humans will be exposed. Uninterpretable results are likely to be obtained if the administration of very high dose levels grossly interferes with the nutritional or hormonal status, etc., of animals.

SYNOPSIS (continued)

At best the results of long-term animal tests provide some guidance in the distinction between substances which are likely to increase cancer risk in humans and those which are unlikely to do so. It is important that every effort is made to obtain meaningful human data especially for persons exposed to chemicals during the manufacture of Cosmetics and Toiletries.

The Evaluation of Cosmetics and Toiletries for Carcinogenicity

Twenty years ago it seemed reasonable to distinguish carcinogens and non-carcinogens on the assumption that they belonged to two completely separate classes. Most chemicals, it was thought are non-carcinogens and only a few are carcinogens. Because they seemed to be so few in number and because most of the known carcinogens were of no commercial interest, a total ban on the use of carcinogens in such ways that workers and/or the general public might be exposed to them, was considered to be realistic. During the last 20 years, developments in epidemiological and laboratory techniques and increased requirements by Regulatory Bodies have completely undermined this concept and we are confronted, firstly, with there being no longer any clear distinctions between carcinogens and non-carcinogens and secondly, with evidence that many chemicals that are in everyday use and are vital to a modern way of life have, under one set of conditions or another, been shown to increase the risk of cancer development in laboratory animals. It is useful to consider some of the developments of the last 20 years. Table I summarises the developments - both the good and the bad. Among the more welcome developments are some of these stemming from Regulatory Bodies. Unquestionably, neither the requirements for tests nor the average standards of testing of 20 years ago were adequate to protect the public from significant exposure to carcinogens, and I outlined some of the common design faults in a paper I presented in Zurich 4 years ago (Roe and Tucker, 1974). The recent evolvements by the U.S. Food and Drug Administration of a code for Good Laboratory Practice is in principle and, apart from certain stupidities of detail, a desirable development. Likewise, it is a great advantage to have readily available, nowadays, stocks of clean animals and laboratories in which long-term studies can be conducted without serious losses through intercurrent disease. On the other hand, it is a matter for serious concern that most of the diets fed to small laboratory animals under test are over-nutritious. The formulae of the standard diets fed to laboratory rats and mice today were worked out to avoid deficiency diseases in animals plagued by various background infections and infestations. These diets are obviously far from optimal for pathogen free animals, as is witnessed by the high incidence of gross obesity which is even more common among laboratory rats and mice than middle-aged Americans. To make matter worse the fat content of standard laboratory diets has, in some cases, actually been increase to avoid the crumbling of pellets during

pasteurization. No one has undertaken the basic research necessary to determine optimal diets for healthy animals destined to live out their natural life-spans. Observation at Imperial Chemicals Industries Laboratories suggest that overnutrition may greatly increase spontaneous tumour incidence (Roe and Tucker, 1974) and observations by Gellatly (1975) indicated that increased dietary fat content magnifies liver tumour incidence in mice.

In the wild, animals are not only plagued by diseases but also have very often to stuggle to find enough nutritious food. The availability ad libitum throughout 24 hours of each day, of an overnutritious diet is a highly unnatural aspect of the conduct of most long term toxicity and carcinogenicity studies today.

Twenty years ago, although the total number of known carcinogens was few, they belonged to a seemingly bewildering variety of chemical types, e.g. polycyclic aromatic hydrocarbons, aromatic amines, azo-dyes, alkylating agents, carbamates, etc. The recognition that relatively inert compounds such as polycyclic aromatic hydrocarbons may be converted to reactive ones with alkylating properties (e.g. epoxides) by a process known as metabolic activation has provided insight into how widely differing chemical structures may come to have similar effects. Understanding has similarly been enhanced by vastly increased knowledge of the consequences of alkylation of nucleic acids and of nucleic-acid repair mechanisms. Although several oncogenic viruses were known earlier, it was not until Ludwig Gross's (Gross, 1951) clear demonstration of the existence of viruses that caused malignant lymphoma (leukaemia) in mice, that the true importance of viruses as determinants of cancer incidence began to be recognised. The role of oncogenic viruses in the apparent induction of cancer by agents is still, in many ways, a frontier-of-knowledge subject. Similarly, although knowledge has accumulated on how hormones act and on how immunological mechanisms may be relevant to cancer development, we are as yet far from achieving a real appreciation of how such factors need to be taken into account in the design and interpretation of carcinogenicity tests on chemical agents.

One thing is certain; however; laboratory animals tend to be highly abnormal from the view point of hormonal status. This is manifested in many ways, including high incidences of pathological changes in the genital tract and high incidences of neoplasms of endocrine glands and hormone-dependent tissues such

as the mammary gland. Lack of stress, lack of exercise, overnutrition and enforced celibacy undoubtedly contribute to the overall abnormality status, but most important may be the fact that male and female animals can smell each other but not mate. The problems created by this aspect of the artificiality of the laboratory enviroment is one that should have received far more attention than it has.

Proceeding down Table I we reach item 4, the development of in vitro methods for screening agents for possible carcinogenicity. It is now possible to show that many agents known to be carcinogenic on administration to intact animals are capable of increasing the risk of malignant transformation of cells maintained in culture. Even today not all investigators are able to achieve positive results with any particular substance. Failure to do so may simply reflect the absence from particular cell cultures of enzymes necessary for metabolic activation. More difficult from the viewpoint of interpretation however, has been the demonstration that malignant transformation by a chemical carcinogen can be greatly facilitated by the presence of known oncogenic viruses (Rhim et al, 1972). Where this is so, it is reasonable to ask which is the more appropriate predictive model system for screening chemicals of unknown carcinogenic potential, - the one with the virus which gives positive results, or the one without the virus which gives an equivocal or negative result? We know too little about the distribution of oncogenic viruses in human tissues to answer this question.

The development during the past few years of sophisticated bacterial mutagenicity screens, such as those with which the name of Dr. Bruce Ames has become associated, has had revolutionary impact. In general terms, these screens pick out chemicals capable of giving rise to either base pair substitution or frame-shift mutations in systems that maximise the possibility of direct contact between the test chemical and nucleic acids, that provide a battery of enzymes that might be needed for metabolic activation and that exclude, as far as possible, the repair of any genetic damage produced by test agents. These screens are extremely sensitive ways of detecting biological alkylating activity of test substances or their metabolics. False negatives can, however, be given if the appropriate metabolically activating enzymes happen to be absent from the battery of such enzymes supplied. A more important problem, however, is that these artificial systems bypass all the

all the normal defense systems of the intact body, with the result that, at best, they predict theoretically possible hazard as distinct from real hazard. As predictions of real hazard they have only limited value. Most authorities have come to accept that bacterial mutagenicity screens have a part to play in the screening of environmental factors for possible carcinogenicity, but that they constitute no adequate substitue for substantive carcinogenicity studies in intact animals.

The availability of computers has opened the way to large scale multi-factorial epidermiological studies which offer the possibility of detecting time/space clusters of cancers and association between exposure to particular environmental factors and particular cancers. One of the most productive kinds of study has been that of migrants from one environment to another, e.g. Japanese who go to live in the U.S.A or Hawaii. These have highlighted the importance of environmental as opposed to genetic factors in the case of cancers of the stomach and colon, (Haenszel & Kurihara, 1968). Similarly, it was epidemiologists conducting large scale surveys, who first firmly established the association between smoking and risk of lung cancer (Doll & Hill, 1954, Hammond & Horn, 1954). However, epidemiological studies unless supported by other evidence can easily lead to false conclusions because of unrecognised biases in the basic data and because, when large numbers of comparisons are being made between, say, groups of cancer subjects and control subjects in respect of exposure to different environmental factors, it is to be expected that every once in a while (e.g. 1 in 20 at the 5% level and 1 in 100 at the 1% level of significance) apparently statistically significant associations will occur by chance. This latter kind of occurrence probably accounts for why reserpine and prolactin-release agents came under suspicion in relation to breast cancer (Leading Article, Lancet 1974). In this instance subsequent studies failed to confirm the suspicion (Leading Article, Lancet 1975).

But cautionary tales like the reserpine story do not prevent the almost daily claim arising from poorly conceived epidemiological studies that some hitherto apparently harmless chemical is an important cause of some form of cancer. Unfortunately, in a world geared to sensationalism, a quick way into the limelight is to make dramatic statements about possible hazards. It is bad enough when this is done by nakedly ambitious politicians, but far worse where

Politicians mascarade as scientists! Consumerism has got itself a bad name with the scientific community because it attracts both cranks and politicians, but Industry should welcome and join rather than fight consumerism with the aim of making it more responsible.

There is no definition of a carcinogen which is at the same time both useful and acceptable to a majority of scientists. Twenty years ago the term was freely applied to any agent which, on administration to animals by any route, increased the incidence of any form of cancer. Thus, materials introduced into animals by a wide variety of routes - some of them quite imaginative to say the least - came to be labelled as carcinogens. The discovery that cancer may arise non-specifically in response to the introduction into tissues of even relatively inert materials and that this effect may be greatly influenced by the physical characteristics and surface characteristics (Grasso & Golberg (1966); Bischoff & Bryson, 1964, Carter et al, 1971) of materials has belatedly led to the realisation that to assess whether a substance is likely to constitute a cancer hazard, it is necessary to take into account the exposure route. The cancer literature is still full of myths stemming from this earlier era of research, although on-going series of Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man prepared by the International Agency for Research on Cancer in Lyon (IARC, 1972-77) has gone some way towards dispelling these myths. In my opinion, "carcinogenicity" is not to be regarded a property of a chemical or other agent. On the contrary, a "carcinogenic effect" should be looked upon as the result of an interaction between the agent in question and a living organism under a particular set of conditions of exposure. A similar interaction may or may not occur between the agent and other living organisms or with the same living organism under different conditions of exposure.

This leads me to stress the essential difference between "testing for carcinogenicity" and "evaluation of carcinogenic hazard". Tests, if properly designed, conducted and statistically analysed, are capable of giving positive or negative results. They cannot by themselves distinguish substances, which under particular conditions of use and exposure, do or do not constitute cancer hazards for man. For this purpose a wide variety of other information is needed and at the end of the day an appraisal based, like a judgement in a civil law case on the balance of probabilities, is all that is possible. Such judgements usually have to be made on evidence that falls far short of what is desirable. Consequently they are necessarily revisable in the light of new evidence. Of

course, the evaluation of hazard does not by itself determine whether or not a compound should be used; assessment of "benefit" and a judgement based on an assessment of all risks relative to all benefits, are necessary for this purpose. Evaluation of carcinogenic hazard does not necessarily depend on the availability of data from animal studies. A consideration merely of the chemical structure or of chemical or epidemiological evidence of cancer hazard in man may be enough to condemn a chemical as being too risky to use. Similarly, a consideration of chemical structure may be enough to satisfy adjudicators that a proposed use of a chemical carries negligible cancer hazard.

Cosmetics and toiletries are basically in the same boat as other chemicals - food additives, food constituents, drugs, pesticides, chemicals used in industry, etc. - in relation to evaluation for carcinogenicity. The fact that more of them are intended for application to the skin or mucous membranes, rather than ingestion, means only that application to the skin must be the more usual route by which such agents are tested for carcinogenicity if tests are needed. The making of risk:benefit judgements is, however, often more difficult because there is no recognised way of assessing benefit. This could become a major problem in the years ahead. Already we see everyday agents such as chloroform, hair-dyes and talc under fire because of suspicion of cancer hazard. How can we assess and express the undoubted benefits of the use of such agents?

I have purposely left until last the vexed question of "dose". The increasing risk of developing cancers of most sites with increasing age possibly reflects the accumulation during life of permanently mutated somatic cells. Even in a completely natural environment such mutations occur in response to sunlight and naturally-occurring mutagens in the diet, etc. Most of the mutations which occur are probably irrelevant to cancer and it may well require the coincidence of several specific mutations for the production of a cell capable of giving birth to a cancer. The healthy body has at its disposal many ways of counteracting the effects of exposure to mutagens. In some cases damaged nucleic acids can be repaired, alternatively damaged cells are selectively shed or attacked by immunological surveillance systems. It is possible that it is more a failure of such defense mechanisms with age rather than the accumulation of mutant cells that determines the increased risk of cancer development in old age. However, one study of our own mice (Peto et al, 1975) did not support this theory.

It is currently assumed by scientists such as Dr. Bruce Ames that evidence of mutation of bacteria is indicative that a chemical is likely to react with mammalian DNA in a way relevant to carcinogenesis. It is also assumed that there is a direct dose-response relationship down to zero dose. At present there is nothing to indicate that this latter assumption is wrong, although instinctively one suspects that it might be. But more importantly, it is likely that, for practical purposes, there are dose-levels for many agents below which exposure would not significantly increase cancer risk above the level necessarily associated with the most pure environment and the most abstemious way of life. A formula was evolved by Mantel & Bryan (1961) for estimating the level of a substance which would be associated with an acceptable degree of risk. They did not recommend what the magnitude of such an acceptable limit of risk should be although in their examples they set it at 1 in 100 million (coincidentally, perhaps, this is equivalent to one extra tumour in the American male population!). If this were the limit, then, for practical purposes all suspect substances would be unusable anyway, since the residues permitted on the basis of the Mantel & Bryan formula would be far less than the sensitivity of methods available for their detection.

I can not help feeling that the prospects facing Cosmetics and Toiletry Manufacturers in relation to the Regulatory Situation in the U.S.A. are gloomy and that the most that can realistically be hoped for is that a somewhat less cautious approach that at present applies to food additives is followed in setting "negligible risk dose-levels" for cosmetics and toiletries.

Another problem is that it is undoubtedly wrong to assume that somatic mutation is the only important mechanism in carcinogenesis. Hormones clearly do not act in this way, nor do tumour-promoters. Also, many of the ways in which co-carginogens act have little or nothing to do with mutation. Thresholds are very likely to exist for the actions of such agents.

The animal tests sponsored by the National Cancer Institute which sparked off the recent flurry of regulatory activity against chloroform, were conducted with levels of chloroform within the lethal range and vastly in excess of those used in medicines or toothpaste. It is possible that at these very high dose levels, a normally effective detoxification mechanism in the liver was swamped, and that at lower doses there would be no risk whatsoever of increased liver tumour incidence

in mice or increase kidney tumour incidence in rats. There is obviously an urgent need for further studies covering the dose range from lethal levels to normal use-levels.

My own evaluation of the pathological material from the NCI study on chloroform left me with an impression which may be of the greatest importance in the future. There was both in the rats and in the mice a striking difference between the high and low dose levels in terms not only of the incidence of tumours, but also of the degree of malignancy of the tumours. This aspect of the findings with chloroform certainly merits further consideration and study.

In this talk I have purposely, if mistakenly, refrained from discussing the details of techniques for testing for carcinogenic activity. There is no shortage of guidance in the open literature with regard to how such tests should be designed and executed. Moreover, the need in future to comply with the standards recommended under Good Laboratory Practice (Federal Register, 1976) will ensure that formerly all-too-frequently errors will be committed less frequently from now on. There remain, of course, may unresolved problems, such as whether tests for carcinogenicity should be terminated after a fixed time or cover the whole of the life span, and whether exposure should start before conception or not until weaning. In my opinion, it would be wrong to develop too rigid an attitude to such questions: flexibility is both necessary and desirable.

S U M M A R Y

The need to distinguish between tests for carcinogenicity and evaluation of carcinogenic hazard is stressed and developments relevant to testing for carcinogenicity discussed. The limited value of bacterial mutagenicity tests as screens for carcinogenicity is acknowledged although such tests are no substitute for properly designed, executed and statistically analysed long-term tests on animals. It is important that animal tests involve an appropriate route of administration and cover a dose range which includes dose-levels of the same order as that to which humans may be exposed. The artificiality of the conditions under which experiments on laboratory animals are necessarily conducted, needs to be taken into account in assessing the significance of the results of such experiments.

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TABLE I

Some Developements in Carcinogenicity Testing during past 20 Years

1. Increased requirements by Regulatory Bodies (i.e. more and better tests).

2. Availability cleaner and healthier animals and better facilities for testing.

3. Increased understanding of:-
   (i) metabolic activation
   (ii) alkylation of DNA
   (iii) DNA repair
   (iv) Oncogenic viruses
   (v) Mechanisms of hormonal action
   (vi) Immunological mechanisms
   (vii) Importance of route of administration
   (viii) Significance of overnutrition

4. Development of screening methods:-
   (i) In-vitro cell transformation
   (ii) Bacterial mutation

5. Advances in computer science enabling large scale epidemiology and multiple variable analysis of epidemiological and experimental data.

6. Encroachment by mathematicians, consumerists and politicians into the field of toxicology.