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Relevance of Short-Term Carcinogenicity Tests to the Study of the Carcinogenic Potential of Urban Air

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It is now accepted that screening for carcinogens in animals is expensive and demonstrates carcinogenic potential rather than actual carcinogenicity in man. A number of short-term tests which depend on mutagenicity, stimulation of DNA repair, ability to produce chromosome damage or other actions, and which correlate at least to some extent with carcinogenic potential, have been devised. These have the advantages of being rapid and cheap. Some can be carried out by using human cells. They may have advantages in the context of air pollution since they are sensitive down to very low dose levels and since they can deal with complex mixtures. Combinations of such tests may be of more value than any single test. Their particular value may be as a preliminary screening procedure in a tiered testing programme which may have high predictive efficiency.

Urban air contains a number of different materials, some of which have been shown to have carcinogenic potential in experimental animal systems. In particular, a number of polycyclic aromatic hydrocarbons have been recognized (*1, 2*). It is not, however, clear whether the presence of these materials is causally related to the excess of malignant disease, particularly of carcinoma of the lung, in urban areas as compared with rural areas. The presence of potential carcinogens is not in doubt. What is not known is whether these compounds are present in a form which is capable of reaching sensitive tissues and, if so, in sufficient quantity to cause cancer. Nor is it known whether the interactions between compounds will affect carcinogenic potential of the individual compounds present in these complex mixtures.

Relevance of Animal Tests

A positive result in an animal carcinogenicity test cannot always be applied directly to the human situation, where the dose and conditions of exposure may be quite different. Normally, in order to keep the numbers of animals within reasonable limits the doses used in animal tests are fairly high. Although a general relationship between dose of carcinogen and response is often apparent, precise measurement of this correlation has proved difficult but in a small, but increasing, number of cases, where a large range of doses is practicable, there is evidence of a precise dose response relationship (*3*). The precise shape of the dose–response curves, especially at the lower levels, is not, however, known and there may, of course, be different curves for different carcinogens. Extrapolation to the low doses of actual exposure is somewhat speculative, though some attempts to do so have given interesting results (*4*).

A second problem is that a compound which gives a positive result in a carcinogenicity test in one species will not necessarily be found to be carcinogenic in another species. Although carcinogens which do not need metabolism are likely to be active to some degree, in all higher species there may still be wide differences as regards sites and degree of activity. Even some compounds of proven carcinogenicity in man have been shown to be carcinogenic in experimental systems only with difficulty, e.g., 2-naphthylamine was shown to be carcinogenic in beagle dogs at relatively large doses (*5*). Even the low dose group was given daily doses of 6.25 mg/kg daily for up to 30 months giving, in

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some cases, a total dose of 30 g. A potent carcinogen in one species may, therefore, appear to be only a weak carcinogen in another and of course the converse may also be true.

It can, therefore, be argued that animal tests do not demonstrate that a compound is a carcinogen which will produce cancer in all species and at all doses but simply a substance with carcinogenic potential which will produce cancers when administered at sufficient dose in an appropriate species. Recently, therefore, attention has focussed on the possibility that there may be other ways of demonstrating that a compound has carcinogenic potential.

Short-Term Tests

A variety of different short-term tests have been devised which, it has been suggested, may be useful as indicators of the potential carcinogenicity of chemical compounds. Some of these are based on detecting mutagenic activity, accepting that a close link exists between mutagenicity and carcinogenicity. Some, however, are concerned with cellular alterations which do not involve mutation or do so only indirectly. The tests are considered in detail in a recent IARC monograph (*6*), but may be summarized as follows:

Mutation-Based Tests

The most widely used are those in which specially developed strains of bacterial cells (*7*) are exposed to a test substance with or without the addition of a liver microsomal extract which can mimic metabolic activation that occurs *in vivo* (*8*). More recently, however, mammalian cells and even human cells have been used for testing mutagenic potential (*9*). The use of human cells, while rather difficult technically, is of particular interest since it removes the need for interspecies extrapolation.

Chromosome Tests

Classical studies of the sort used for elucidating dose response relationships for x-rays must be used but these have proved insensitive indicators of *in vivo* chemical exposure in the system studied so far. More recently techniques have been developed for measuring the frequency of exchanges between sister chromatids in mammalian cells (*10*). The sensitivity of this test may be quite exceptional, since it has been shown that cells from patients with xeroderma pigmentosum may be especially susceptible to the induction of damage of this kind by chemical agents (*11*).

DNA Repair

Measurement of degree to which repair of DNA is stimulated after exposure to chemical agents has been shown to correlate well with carcinogenic potential (*12*). These tests can also be done with bacterial cells and again appear to be very sensitive.

Mutation of Multicellular Organisms

The mouse dominant lethal test is thought to have a number of disadvantages compared with the cellular tests. Very large numbers of animals are required and even then evaluation of the results is complex (*13*). The use of specific locus mutation tests in Drosophila is also being explored and may offer some advantage.

Cellular Transformation

Transformation of mammalian cells by chemicals has proved to be difficult compared with transformation by viruses. Most workers would agree that this is not suitable for screening for potential carcinogens, but Purchase et al. (*13*) have claimed that a system of detecting transformed cells by cloning in soft agar is useful in detecting carcinogens.

Other Tests

The degranulation of microsomes has been suggested as an indicator of carcinogenicity (*14*) but, while it has been shown to be useful in some cases, it is not effective with all classes of carcinogens. Similarly, the observation of biphenyl 2-hydroxylation may be useful to indicate some classes of carcinogens (*15*). It seems clear that no one of these tests will give a perfect correlation with carcinogenicity but combinations of tests may have a good predictive value. More particularly it has been suggested that a tiered system of testing including these tests may be a practical way to assess carcinogenic risks of environmental contaminants (*16, 17*).

Possible Areas of Usefulness in Considering Urban Air Pollution

Low Doses

The two major problems in trying to apply the results of animal tests to the assessment of risks of human exposure are the difficulties of extrapolation from high experimental doses and the necessity to transpose results from one species to another, in the

knowledge that even in animal tests the response of different species may be radically different. The use of the procedures outlined above may be helpful in both areas. The activity of compounds known to be present in urban air could be tested directly on human cells by use of cell mutation, sister chromatid exchange, or stimulation of DNA repair as indicators. Although the use of human cells is not yet commonplace, the technology is not difficult and the method could easily be more widely used. Further, one could reasonably expect activity in those tests at dose levels approaching the actual levels of exposure. For example, Wolff et al. (*11*) reported a measurable increase in sister chromatid exchanges in cells from patients with xeroderma pigmentosum following exposure to $4 \times 10^{-10}M$ 2-nitroquinoline oxide or $10^{-9}M$ mitomycin C. Moreover, these same results show a good dose response relationship. Similarly, the bacterial mutagenicity tests are extremely sensitive, and mutations can be measured as revertants per nanomole of chemical (*8*).

Mixtures

While such tests may be useful for studying individual compounds, they may be more useful, especially in the context of the present problem, for studying mixtures. Urban air contains many different substances; many of these, like the polycyclic hydrocarbons, are recognized carcinogens, while others, such as some phenols, have cocarcinogenic activity. However, many of the substances have not been adequately tested, and since air in different locations will vary, any particular sample may also contain many unknown compounds. The process of identifying and testing all of the compounds individually would be formidable; attempts to circumvent this problem by preparing and testing condensates, which are partial mixtures, are not entirely satisfactory. The technical nature of some of the short-term tests, particularly the bacterial mutagenicity tests, is such that testing of more representative mixtures may be possible. The biggest problem in putting these ideas into practice is the inevitable microbial contamination of air. Most means of sterilizing air would also be likely to affect the chemical composition. Filtration, while removing particulates and some of the condensable material, could, however, allow the direct evaluation of the volatile constituents using techniques that have already been used for volatile carcinogens such as vinyl chloride (*18*). The particulate material retained on the filter could also be tested directly. Indeed, one recent report shows not only that this can be done but, also that particulates from urban air show considerable biological activity. Tokiwa et al. (*19*) found that in a bacterial mutagenicity test, some samples of air yield over 100 mutants per cubic meter of air. They also measured the various components of the air tested and concluded that the mutagenic activity cannot be explained by the presence of polycyclic aromatic hydrocarbons alone. While ultimately the recognition of the specific active components is desirable, it is more important in the short run simply to demonstrate whether or not the mixture that is urban air has carcinogenic potential.

New Contaminants

When a new source of atmospheric pollution arises, it could take a quite unreasonable time for all the contaminating materials to be recognized and for them to be tested adequately for carcinogenic activity. In such a situation, the short-term tests could be used and indeed might be the only way to monitor the atmosphere. Even if it cannot be accepted that a positive result in such a test necessarily shows that the new pollutant constitutes a cancer hazard, it should be accepted as an indication for further studies, possibly in an agreed tiered system. The early warning that such studies could give might help in reducing pollution as soon as possible and serve to prevent human morbidity. The use of these tests in other screening situations has met with some opposition, largely on the grounds that the correlation between positive tests and proven carcinogenicity is in all cases less than perfect. In some cases compounds known to produce tumors in animals are only weakly positive in a mutagenicity test, e.g., the nitroso compounds in the bacterial mutagenicity test (*8*). Similarly, some compounds which appear to be only weak carcinogens give strong positives, e.g., mitomycin C in the sister chromatid exchange test (*11*). However, if one recalls that animal tests themselves can give results which may be misleading so far as man is concerned and that they demonstrate only potential for causing cancer, the other tests are seen in better perspective. If they are considered only as indicators, as part of a system rather than as a final assessment of risk, they will have a useful role to play.

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