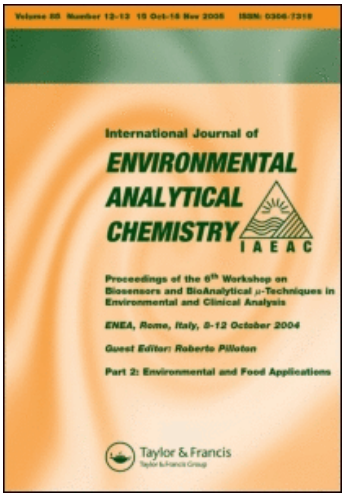

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The Application of Mutagenicity Tests to the Determination of Water Quality†

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It is now known that our life-style, which includes what we drink, is the largest single factor contributing to the type of cancer we are likely to incur. Experimental evidence supports this observation in that rodents can have significantly different "spontaneous" tumour rates depending solely on their growth environment. In both man and animals drinking water quality has been postulated as being a contributing factor and indeed some potable waters have been found to possess mutagenic activity, at least towards bacteria. Such speculation has led to the call for stricter governmental control for the quality of drinking water, and attempts have been made to establish a quality specification for water based on bacterial mutagenicity assays. It is our view that this is impossible because, (1) bacterial mutagenicity tests are in themselves erroneous predictors of carcinogens, and (2) it is only after the mutagenic agent in water has been characterised that risk assessments could be made and appropriate action taken. Legislation based solely on hazard identification is not a realistic method of management since it must assume equality of carcinogenic potency for all mutagenic agents.

KEY WORDS: Drinking water, mutagenicity, carcinogenicity, predictive tests.

INTRODUCTION

The planet on which we live, the air we breathe, the water we drink and the food we eat are all contaminated to some extent with the

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products of human activities, and the chemical industry is aware that certain of its raw materials, by-products and products, can reach the aquatic environment by various routes. It is concerned to play its part to ensure that the highest standards are maintained in the quality of our daily water supply and to co-operate in the identification and removal of possible causes of cancer. However, the effects of low levels of these pollutants on the incidence of chronic diseases such as cancer are difficult, if not impossible, to measure directly, since many different pollutants are likely to be present in each geographic location and the absolute risks from each are likely to be low when compared to the risks of smoking, drinking, overeating or some other enjoyable, even if harmful, habits. In addition, evidence from trend analysis of cancer mortality over the last 10 years does not suggest any major new hazard has been introduced to the environment in the preceding decades other than the well recognized hazard of cigarette smoking. Indeed, contrary to popular belief, most of the trends in recorded mortality of people under 65 are downward and for those under 45 they are particularly favourable.[1] It is important to emphasise this point because this mistaken popular belief may in turn be a cause of mistaken priority setting in the campaign for better cancer prevention.

The estimates of risk attributable to different classes of environmental agents are recorded in Table I, the term "life-style" meaning factors "such as lack of dietary fibre, excess fat and calorific intake, and possibly hormonal carcinogenesis".[2]

The situation with regard to drinking water pollution is more obscure. Because analytical techniques now permit the detection of some laboratory carcinogens at levels below 1 ppb, and because some carcinogens have been identified in drinking water (Table II) the EPA has promulgated a regulation in the U.S.A. establishing a "maximum contaminant level" of 100 μg total trihalomethanes per litre for all community water systems serving 10,000 persons or more and adding disinfectant in their treatment of water supplies. The EPA did not claim that drinking water caused cancer but conceded that because human exposure may be prolonged, that even such weak stimuli may in principle have adverse health effects. The evidence that this may be the case is derived from (1) the activity of certain pollutants, e.g. chloroform, in animal cancer bioassays, (2) that some pollutants are known or believed to cause cancer in

TABLE I

The proportions of cancer deaths attributed to various factors.[a]

Contributing factor	Percent of all cancer deaths	
	Best estimate	Range of acceptable estimates
Tobacco smoking	30	25–40
Alcoholic drinks	3	2–4
Diet	35	10–70
Food additives	<1	−5–2
Reproductive and sexual behaviour	7	1–13
Occupation	4	2–8
Environmental pollution	2	<1–5
Industrial products	<1	<1–2
Medicines and medical procedures	1	0.5–3
Geophysical factors	3	2–4
Infection (parasites and viruses)	10?	1–?

[a]Adapted from Higginson and Muir.[2]

TABLE II

Results from the EPA analysis of representative contaminants in an 80-city survey.[a]

Compound	Number of cities detected in	Range of concentration (μg/l)
Chloroform	80	<0.1–311
Bromodichloromethane	78	0.3–116
Dibromochloromethane	72	<0.4–100
Bromoform	26	<0.8–92
Carbon tetrachloride	10	<2–3
1,2-Dichloroethane	26	<0.2–6

[a]For details see Harris *et al.*[19]

humans if large amounts are ingested e.g. asbestos and VCM, and (3) that there are human population studies that report positive correlations between the amounts of contaminants and certain cancers.[3] Of particular interest is the claim that there is a positive relationship between the concentration of halogenated organic matter in water (derived from sterilisation by chlorination) and mortality from cancers of the bladder and large intestine.[4] Many specific halogenated compounds, ($CHCl_3$, CCl_4, VCM, CH_2Cl_2 etc.) have been found to affect the cancer incidence in experimental animals and it is therefore reasonable to ask whether life-long exposure to the non-specific mixture present in chlorinated drinking water has any material effect on the risks for man, no matter how small that risk may be, relative to, for example, air pollution.

Rapid tests for the identification of potential carcinogens

Certain *in vitro* tests (the Ames Test in particular) are quick and inexpensive methods of identifying possible mutagens and carcinogens. They are a most important development and add to our capability of predicting hazard and they reduce the need for animal testing, but they are still research tools which require expert conduct and interpretation.

The Ames Salmonella reverse mutation test,[5] is the most popular by far. The principle is as follows:

Mutants of Salmonella which require histidine for growth are exposed to the test chemical in the absence or presence of rat liver post-mitochondrial supernatant (S9-mix) to simulate mammalian metabolism. If the test material is a mutagen, or if it is capable of being metabolised to a mutagen, then some of the exposed Salmonella will be genetically modified so that they will be able to grow without added histidine in their medium. After 2–3 days incubation, the number of colonies of revertants are determined and a dose-response curve constructed. A positive response, i.e. a mutagenic event, is generally accepted as one which produces a reproducible two-fold increase in mutation frequency with a dose-response relationship.[6]

Since Ames publication in 1973[7] that "Carcinogens are mutagens, a simple test system combining liver homogenates for activation and bacteria for detection...", there have been several validation studies

aimed at determining the predictive value of the test as compared with other short-term predictive tests. Purchase *et al.*,[8,9] published their findings which like others in the field, found Ames test to be about 90% predictive. However, even then, it was found that some modifications were required to the "plate incorporation" protocol to identify such materials as vinyl chloride monomer, methyl chloride and monochlorodifluoromethane, (by testing in the vapour phase) and dimethylaminoazobenzene (by testing as a pre-incubation or fluctuation assay) as bacterial mutagens. In addition the significance of competent S9-mix in the detecting of human carcinogens such as 3,4-benzo(a)pyrene by *Salmonella* was exemplified by Oesch *et al.*[10] Also there are now recognized to be many experimental carcinogens not identifiable by Ames test alone e.g. safrole, hexamethyl-phosphoramide, $CHCl_3$ etc., and as a result of several retrospective validation studies in which known carcinogens and non-carcinogens were screened blind in a variety of short-term predictive tests, it is now recognised that no single test is capable of providing an adequate screen for unknown carcinogens. Nevertheless, despite these difficulties, Ames test in general has become recognised as a cheap, rapid and effective test for identifying potential carcinogens, and numerous regulatory authorities now require the results of this test (or a similar one) for pre-marketing (EEC) or pre-manufacturing (EPA) notification programmes. However, it is recommended that tests for point mutation, chromosome damage, and tests for DNA damage and its repair are necessary for identifying hazardous chemicals and mixtures as potential carcinogens. For the most part however, as far as water testing is concerned, only tests involving point mutation in bacteria, and specifically Ames tests, have been employed.

Ames test as applied to aqueous samples

Having identified some of the strengths and weaknesses of Ames test as a general predictive bioassay for known chemical carcinogens, it is interesting to review how well it shapes up to water and beverage testing. Some recent observations are as follows:

1) In Holland, Sloof and Van Kriejl[11] have examined the waters from the Meuse and Rhine and when concentrated to about 1×10^3 they have been found it to be mutagenic. In addition, S9-mix

prepared from locally caught fish is more active in metabolising genotoxic agents than fish caught elsewhere. The significance of these findings for hazard assessment to man is unclear but they bear serious consideration simply as an observation.

2) Meier *et al.*[12] have found that potent mutagens can be produced by the chlorination of humic acid, but in these cases, S9-mix caused a reduction of the activity to normal levels. It could be argued, as pointed out by Sugimura[13] when talking about mutagens in cooked foods, that this reflects the way in which the intact mammal detoxifies mutagens and is thus immune from genotoxic damage from such materials.

3) Tabor and Loper,[14] have reported that concentrated samples (about 5000 ×) of Cincinnati drinking water are mutagenic in Ames test to about the same degree as the known human carcinogen, β-naphthylamine, the implication being that not only are potential carcinogens present in drinking water, but that because their mutagenic potency is of the same order of magnitude as known human carcinogens, this concentrate would be equally potent as a carcinogen to man. In contrast, however.

4) Stolz *et al.*[15] have found that ×3 concentrates derived from such popular beverages as red wine, grape juice and coffee are mutagenic to Salmonella. These mutagens are thus several times more potent than water contrates, and reflect the view that diet per se is probably much more significant as a contributor to life style and cancer burden than drinking water. Here, however, are also problems associated with the concentration of materials which can lead to false positive results being generated.

To summarise thus far, it is clear that geographic location, genetic predisposition, and life style are major determinants in the human cancer burden, and if the exact causes could be identified and removed, there is the theoretical possibility that 90% of cancers could be eliminated. There is also no doubt that some cancers have been industrially induced, β-naphthylamine, benzidine, VCM, etc. are examples of such occupational carcinogens, all of which can be detected by short-term tests. Taken in isolation, Ames test is about 90% predictive but there are several practical considerations which detract from its general applicability and it can lead to false positive results which if over interpreted can cause alarm to the general

public, especially when related to ubiquitous commodities like drinking water.

Other predictive tests for genotoxicity

Other short-term predictive tests are needed to complement Ames test results on water concentrates. There have been numerous options put forward for such bioassays but the general procedure adopted by most parties is reflected in the scheme recommended by Ashby.[16]

The procedure indicates that genotoxicity data must be obtained initially *in vitro* with both point mutation and chromosome analysis being obtained. Negative i.e. non-mutagenic results in these systems probably suggest safety since they are if anything ultra-sensitive to genotoxins. Positive results, however, need to be explored further by measuring genotoxic damage *in vivo* e.g. by recognising repair to DNA damage in the livers of rodents challenged by test materials or by chromosomal effects recognised by dominant lethal effects or bone-marrow damage.

Finally, carcinogenicity can only be truly measured by long term bioassays and such studies must be undertaken with short-term test positive samples to establish base lines for procedures and to establish the carcinogenic potency of such mutagenic samples. The direct extrapolation of Ames test results to animal carcinogenic potency as proposed by Meselson and Russell[17] has not been universally adopted because of the large number of exceptions to their correlation. Health risk assessments can only be derived with confidence from conventional animal studies which clearly demonstrate a no-effect level. An agreed safety factor can then be applied and the data extrapolated to man.

It is perhaps instructive to anticipate the results of such a proposed chronic toxicity study in rodents and to calculate to the added risk of cancer to man from drinking the water sample using the linear one-hit model.[18] Let us assume that the incidence of a particular tumour were to be increased in a dose related manner in a study in which rats were dosed a range of water concentrates, up to 500-fold, for two years. A not unrealistic response could be that at the highest dose level, i.e. 500 × concentrate, a control tumour incidence of 1 in 100 was increased to 10 in 100. (These figures are

within the sort of range of "spontaneous" tumour incidence seen in historical control animals in toxicology laboratories and for the purposes of this argument are ascribed to variations in water quality between experiments. In actual fact, the real reasons for this variation in spontaneous tumour incidence as yet cannot be ascribed to any particular causative agent).

Now, since the total water intake in animals is proportionally similar on a surface area basis, because man is 500 times larger than the rats, then the $500\times$ concentrate dosed to the rats in considered equivalent to neat water in man. If we assume that tap water represents 1/10 total human water intake then according to the model the excess cancer risk is calculated to be 2×10^{-5} and in the more unlikely case if all water intake, i.e. including that in food, is contaminated the risk is calculated to be 2×10^{-4}.

To put these figures into the context of current regulatory thinking, the added risk of cancer generally recognised as safe, i.e. acceptable risk, is 1×10^{-6}; and therefore our hypothetical results observed in rats assumed to be due to the concentrated drinking water would be considered marginally carcinogenic and to just represent the risk of cancer induction in man. In other works, it is just conceivable on the basis of current knowledge of spontaneous tumour variation in rodents that drinking water concentrates could be shown to be marginal carcinogens to animals and could be acknowledged as potentially weak carcinogens to man.

However, on the basis of Ames test, alone, if we accept the Meselson and Russell data[17] as meaningful and back extrapolate the mutagenic potency to carcinogenic potency we see that the current results from Ames tests on water or fruit juice concentrates represent a much more serious threat of cancer in the same order of magnitude as benzidine or β-naphthylamine, materials known to be very potent carcinogens to animals and man.

Clearly, since our experience with water and grape juice is not one of extreme carcinogenicity, there is a need to generate more data before we allow ourselves the luxury of concern over mutagenicity tests and water quality in relation to risk to human health.

CONCLUSIONS

The testing of water samples for mutagenic activity (Ames testing) has

performed a useful role in the assessment of water quality (particularly drinking water) since it has highlighted the presence of mutagens and encouraged the determination and measurement of chemicals at very low levels in water.

It is agreed that exposure to mutagens is to be avoided wherever possible, not only because of the link with cancer but possibly more importantly, because of the more insidious and potentially disastrous effect on the human gene pool.

However, the continued wide application of this test for the control of water quality is of more dubious value; the detection of mutagenic activity has raised emotions while the risk to man of continued consumption of mutagens at very low concentrations remains very difficult to assess in relation to other risks.

The detection of mutagenic water samples is only a preliminary step in potential hazard identification. Any water source that has repeatedly given Ames positive results requires further testing in order to demonstrate carcinogenic potency.

Thus identification of potentially carcinogenic substances is not the problem. The problem is to find a method to identify the level of exposure that represents a harmful concentration.

In effect the procedure requires that the water source should be subjected to the same testing procedures that are applied to individual chemicals of economic importance (drugs, pesticides etc.). Whole animal studies should be an integral part of the programme and the whole package would probably be very expensive. However, without these data it is impossible to predict the hazard to consumers. A risk assessment could then be made with the generated data and it is then, and only then, that any risk can be rationally quantified and managed.

The alternative approach towards the resolution of this difficult problem would be the continued search for the individual organic chemicals which make up the organic carbon in mutagenic water samples. Many of these micro-pollutants are already identified and the ability of analytical chemists to detect and identify organic molecules is steadily improving. Often the carcinogenic potency of synthetic chemicals occurring in water is already known and additional compounds are continuously being tested. Although the determination of synergistic effects of low concentrations or carcinogenic chemicals requires further resolution, the risk assessment of

mutagenic samples could be best undertaken on a more rational basis and the assessment could perhaps be applied to the majority of mutagenic water samples.

Although there is no evidence for the presence of known human carcinogens in major drinking water supplies at concentrations sufficient to account for a significant percentage of the total cancer risk,[1] their detection at low levels could be interpreted as a possible health hazard. However, until the risk from such carcinogenic/mutagenic chemicals can be quantified, the need for their removal cannot be assigned any priority compared to other better defined areas of environmental risk. Since financial resources are always limited, they must be allocated to those areas which will bring about the most benefit to man.

It is our opinion that Ames test has a place in the assessment of water quality but we should not be bullied by enthusiastic regulators (and academics) into believing that Ames positive concentrates reflect public danger, or conversely that Ames negative water is perfectly safe. To paraphrase Sugimura[13] "let us be cautious about alarming the public unnecessarily and go about our business collecting scientific data in a rational manner".

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