Appendix 1

The concept of activity profiles of antimutagens

To facilitate an analysis of data from the open literature on antimutagenicity in short-term tests, we have applied the concept of activity profiles already used successfully for mutagenicity data (Waters *et al.*, 1988, 1990) to antimutagenicity data. The activity profiles display an overview of multi-test and multi-chemical information as an aid to the interpretation of the data. They can be organized in two general ways: for mutagens that have been tested in combination with a given antimutagen or for antimutagens that have been tested in combination with a given mutagen (Waters *et al.*, 1990). The profile presented here is an example of mutagens that have been tested in combination with a single antimutagen and they are arranged alphabetically by the names of the mutagens tested. These plots permit rapid visualization of considerable data and experimental parameters, including the inhibition as well as the enhancement of mutagenic activity. A data listing, arranged in the same order as the profile, is also given to summarize the short-term test used, the doses of mutagens and antimutagens, the response induced by the antimutagens, and the relevant publications.

The antimutagenicity profile graphically shows the doses for both the mutagen and antimutagen and the test response (either inhibition or enhancement) induced by the antimutagen. The resultant profiles are actually two parallel sets of bar graphs (Figure 1). The upper graph displays the mutagen dose and the range of antimutagen doses tested. The lower graph shows either the maximum percent inhibition represented by a bar directed upwards from the origin or the maximum percent enhancement of the genotoxic response, represented by a bar directed downwards. A short bar drawn across the origin on the lower graph indicates that no significant (generally < 20%) difference in the response was detected between the mutagen tested alone or the mutagen tested in combination with the antimutagen. Codes used to represent the short-term tests in the data listings have been reported previously (Waters *et al.*, 1988), and the subset of tests represented in this paper are shown in the Appendix.

In assembling the data base on antimutagens and presumptive anticarcinogens, the literature was surveyed for the availability of antimutagenicity data (Waters *et al.*, 1990), and publications were selected that presented original, quantitative data for any of the genotoxicity assays that are in the scope of the genetic activity profiles (Waters *et al.*, 1988).

The same short-term tests used to identify mutagens and potential carcinogens are being used to identify antimutagens and potential anticarcinogens. The tests are generally those for which standardized protocols have been developed and published. Many of these tests have been evaluated by the USEPA Gene-Tox Program (Waters, 1979; Green and Auletta, 1980; Waters and Auletta, 1981; Auletta *et al.*, 1991) or the National Toxicology Program (Tennant *et al.*, 1987; Ashby and Tennant, 1991) for their performance in detecting known carcinogens and noncarcinogens or known mutagens and nonmutagens (Upton *et al.*, 1984; Waters *et al.*, 1994).

It is not clear at the present time whether antimutagenicity observed in short-term tests is a reliable indicator of anticarcinogenicity since the available data are incomplete. Information on both antimutagenicity and anticarcinogenicity in vivo for a number of chemical classes is required before such a conclusion can be drawn. Clearly, antimutagenicity tests performed *in vitro* will not detect those compounds that act in a carcinogenicity bioassay *in vivo*, for example, to alter the activity of one or more enzyme systems not present *in vitro*. Rather, the in-vitro tests will detect only those compounds that inhibit the metabolism of the carcinogen directly, react directly with the mutagenic species to inactivate them or

otherwise show an effect that is demonstrable *in vitro*. Thus, it is essential to confirm putative antimutagenic activity observed in vitro through the use of animal models. Indeed, the interpretation of antimutagenicity data from short-term tests must be subjected to all of the considerations that apply in the interpretation of mutagenicity test results. Moreover, the experimental variable of the antimutagens used must be considered in addition to the variables of the mutagens and short-term tests used. Obvious examples of parameters that must be considered in evaluating results from short-term tests *in vitro* are: (1) the endpoint of the test, (2) the presence or absence of an exogenous metabolic system, (3) the inducer that may have been used in conjunction with the preparation of the metabolic system, (4) the concentration of S9 or other metabolic system used and whether that concentration has been optimized for the mutagen under test, (5) the relative time and order of presentation of the mutagen and the antimutagen to the test system, (6) the concentration ratio of the mutagen relative to the antimutagen, (7) the duration of the treatment period, and (8) the outcome of the test, i.e. inhibition or enhancement of mutagenicity. Similar considerations apply to the evaluation of in vivo tests for antimutagenicity.

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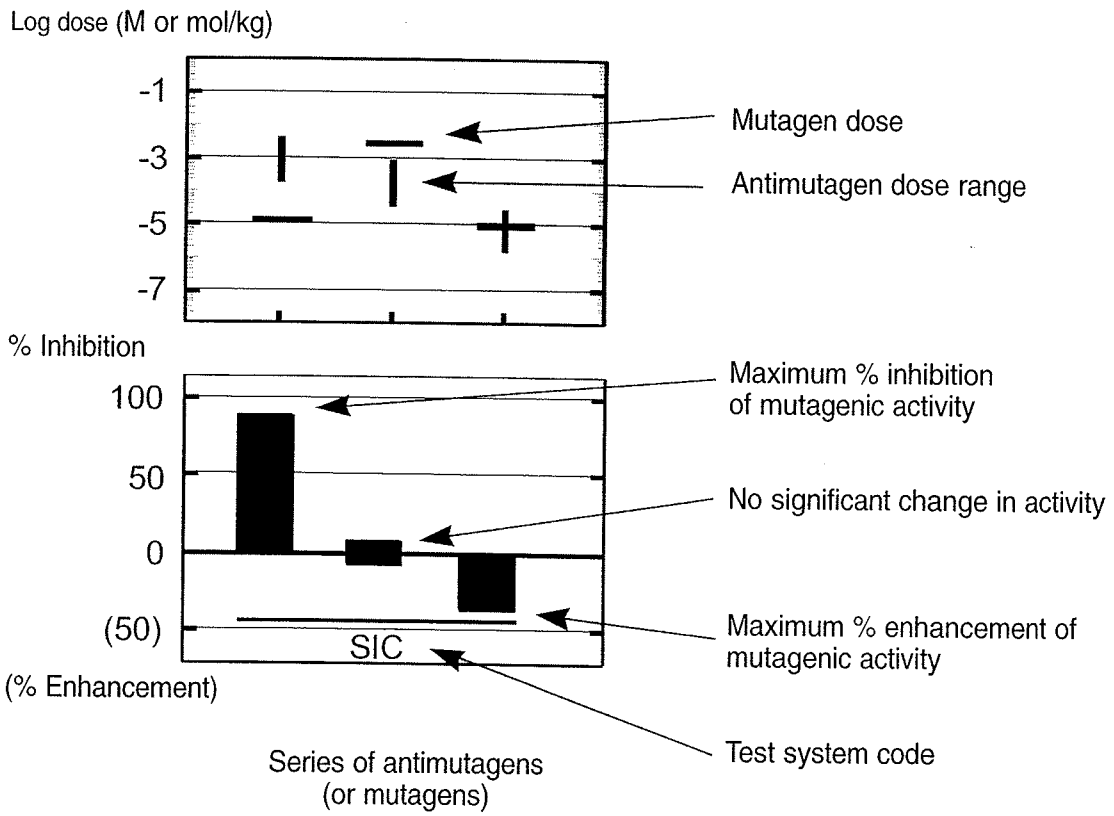
Figure 1. Schematic diagram of an antimutagenicity profile. Profiles are organized to display either the antimutagenic activity of various antimutagens in combination with a single mutagen or the activity of a single antimutagen with various mutagens. The upper bar graph displays the mutagen concentration and the range of antimutagen concentrations tested. The lower graph shows either the maximum percent inhibition, represented by a bar directed upwards from the origin, or the maximum percent enhancement of the genotoxic response, represented by a bar directed downwards. As illustrated in the lower graph, a bar across the origin indicates that no significant (< 20%) effect was detected (designated as 'negative data' in the text). Test codes are defined in Appendix 2.



Appendix 2

Definitions of test codes

Test	Definition
AIA	Aneuploidy, animal cells *in vitro*
AIH	Aneuploidy, human cells *in vitro*
BID	Binding (covalent) to DNA *in vitro*
BSD	*Bacillus subtilis rec* strains, differential toxicity
CBA	Chromosomal aberrations, animal bone-marrow cells *in vivo*
CHF	Chromosomal aberrations, human fibroblasts *in vitro*
CHL	Chromosomal aberrations, human lymphocytes *in vitro*
CIC	Chromosomal aberrations, Chinese hamster cells *in vitro*
CVA	Chromosomal aberrations, other animal cells *in vivo*
DIH	DNA strand breaks, cross-links or related damage, human cells *in vitro*
DMX	*Drosophila melanogaster*, sex-linked recessive lethal mutation
ECK	*Escherichia coli* K12, mutation
G9H	Gene mutation *hprt* locus, Chinese hamster V79 cells *in vitro*
GIA	Gene mutation, other animal cells *in vitro*
HMM	Host mediated assay, microbial cells in animal hosts
MVM	Micronucleus formation, mice *in vivo*
SA0	*Salmonella typhimurium* TA100, reverse mutation
SA5	*Salmonella typhimurium* TA1535, reverse mutation
SA7	*Salmonella typhimurium* TA1537, reverse mutation
SA8	*Salmonella typhimurium* TA1538, reverse mutation
SA9	*Salmonella typhimurium* TA98, reverse mutation
SCG	*Saccharomyces cerevisiae*, gene conversion
SHL	Sister chromatid exchange, human lymphocytes *in vitro*
SHT	Sister chromatid exchange, transformed human cells *in vitro*
SIC	Sister chromatid exchange, Chinese hamster cells *in vitro*
SIM	Sister chromatid exchange, mouse cells *in vitro*
SIT	Sister chromatid exchange, transformed cells *in vitro*
SLH	Sister chromatid exchange, human lymphocytes *in vivo*
SPM	Sperm morphology, mouse
TCM	Cell transformation, C3H 10T1/2 mouse cells
URP	Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*