Appendix 1

Interconversion of units for vitamin A

1 mmol retinol	=	286 mg
1 mmol retinal	=	284 mg
1 mmol retinoic acid	=	300 mg
1 mmol retinyl acetate	=	328 mg
1 mmol retinyl palmitate	=	524 mg
1 mg all-*trans*-retinol	=	3.33 International Units (IU) of retinol or retinyl esters
		3.5 nmol all-*trans*-retinol
		1 μg retinol equivalents (RE)
		6 μg all-*trans*-β-carotene
		12 μg other all-*trans* provitamin A carotenoids

Appendix 2

Three-letter test codes used in activity profiles

Code	Definition
BID	Binding (covalent) to DNA *in vitro*
BVD	Binding (covalent) to DNA, animal cells *in vivo*
BVP	Binding (covalent) to RNA or protein, animal cells *in vivo*
CBA	Chromosomal aberrations, animal bone marrow cells *in vivo*
CCC	Chromosomal aberrations, spermatocytes treated and observed *in vivo*
CIC	Chromosomal aberrations, Chinese hamster cells *in vitro*
DIA	DNA strand breaks, cross-links or related damage, animal cells *in vitro*
DVA	DNA strand breaks, cross-links or related damage, animal cells *in vivo*
GCO	Gene mutation, Chinese hamster ovary cells *in vitro*
GIH	Gene mutation, human cells *in vitro*
G9H	Gene mutation, Chinese hamster lung V-79 cells, HPRT locus
MVM	Micronucleus test, mice *in vivo*
MVR	Micronucleus test, rats *in vivo*
SAD	*Salmonella typhimurium*, differential toxicity
SA0	*Salmonella typhimurium* TA100, reverse mutation
SA2	*Salmonella typhimurium* TA102, reverse mutation
SA4	*Salmonella typhimurium* TA104, reverse mutation
SA5	*Salmonella typhimurium* TA1535, reverse mutation
SA8	*Salmonella typhimurium* TA1538, reverse mutation
SA9	*Salmonella typhimurium* TA98, reverse mutation
SCF	*Saccharomyces cerevisiae*, forward mutation
SCR	*Saccharomyces cerevisiae*, reverse mutation
SHL	Sister chromatid exchange, human lymphocytes *in vitro*
SIC	Sister chromatid exchange, Chinese hamster cells *in vitro*
SVA	Sister chromatid exchange, animal cells *in vivo*
TCM	Cell transformation, C3H $10T_{1/2}$ mouse cells
UIA	Unscheduled DNA synthesis, other animal cells *in vitro*
URP	Unscheduled DNA synthesis, rat primary hepatocytes

Appendix 3

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 database 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 & Auletta, 1980; Waters & Auletta, 1981; Auletta *et al.*, 1991) or the National Toxicology Program (Tennant *et al.*, 1987; Ashby & 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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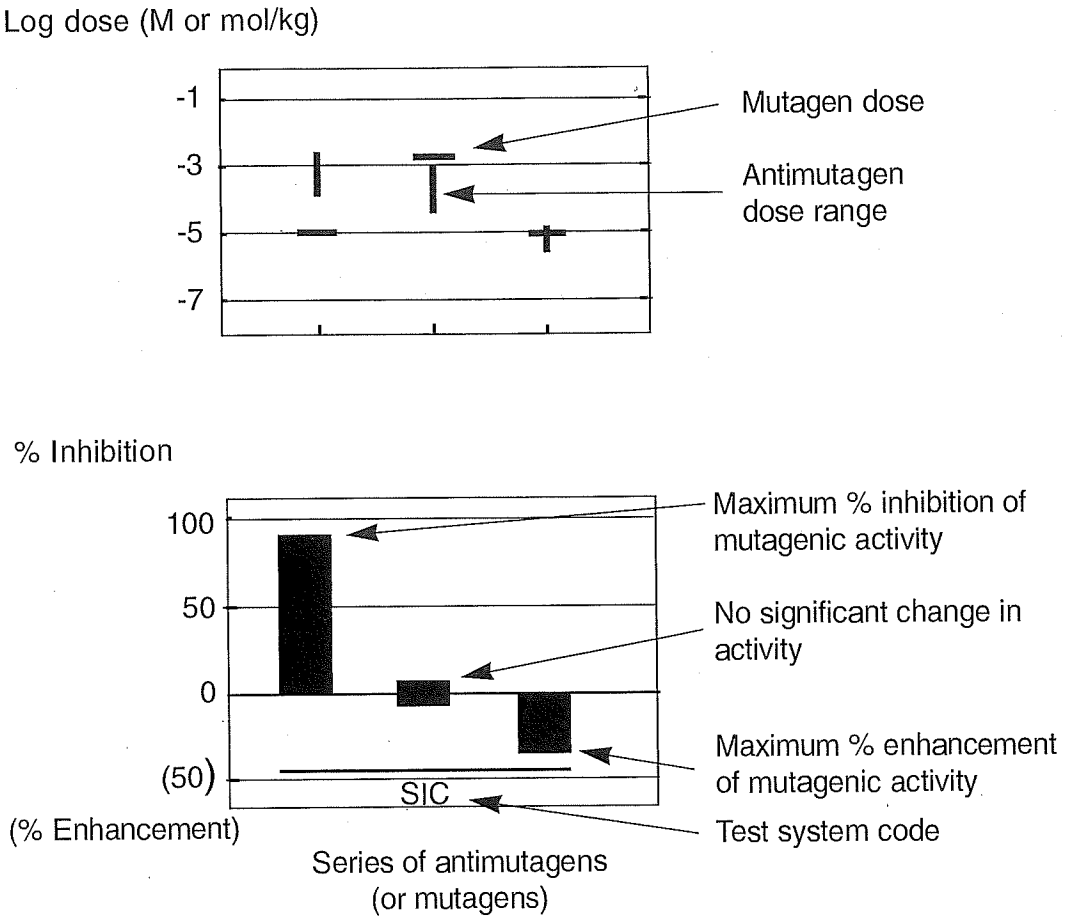
Figure 1. Schematic diagram of an antimutagenicity profile. Profiles are organized to display either the antimutagenic activity of various antimutagens in combination with a single mutagen or the activity of a single antimutagen with various mutagens. The upper bar graph displays the mutagen concentration 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 4

Activity profiles for genetic and related effects

Methods

The *x*-axis of the activity profile (Waters *et al.*, 1987, 1988) represents the bioassays in phylogenetic sequence by end-point, and the values on the *y*-axis represent the logarithmically transformed lowest effective doses (LED) and highest ineffective doses (HID) tested. The term 'dose', as used in this report, does not take into consideration length of treatment or exposure and may therefore be considered synonymous with concentration. In practice, the concentrations used in all the in-vitro tests were converted to μg/mL, and those for in-vivo tests were expressed as mg/kg bw. Because dose units are plotted on a log scale, differences in the relative molecular masses of compounds do not, in most cases, greatly influence comparisons of their activity profiles. Conventions for dose conversions are given below.

Profile-line height (the magnitude of each bar) is a function of the LED or HID, which is associated with the characteristics of each individual test system — such as population size, cell-cycle kinetics and metabolic competence. Thus, the detection limit of each test system is different, and, across a given activity profile, responses will vary substantially. No attempt is made to adjust or relate responses in one test system to those of another.

Line heights are derived as follows: for negative test results, the highest dose tested without appreciable toxicity is defined as the HID. If there was evidence of extreme toxicity, the next highest dose is used. A single dose tested with a negative result is considered to be equivalent to the HID. Similarly, for positive results, the LED is recorded. If the original data were analysed statistically by the author, the dose recorded is that at which the response was significant ($p < 0.05$). If the available data were not analysed statistically, the dose required to produce an effect is estimated as follows: when a dose-related positive response is observed with two or more doses, the lower of the doses is taken as the LED; a single dose resulting in a positive response is considered to be equivalent to the LED.

In order to accommodate both the wide range of doses encountered and positive and negative responses on a continuous scale, doses are transformed logarithmically, so that effective (LED) and ineffective (HID) doses are represented by positive and negative numbers, respectively. The response, or logarithmic dose unit (LDU_{ij}), for a given test system *i* and chemical *j* is represented by the expressions

$$LDU_{ij} = -\log_{10} (\text{dose}), \text{ for HID values; } LDU \leq 0$$

and

$$LDU_{ij} = -\log_{10} (\text{dose} \times 10^{-5}), \text{ for LED values; } LDU \geq 0.$$

These simple relationships define a dose range of 0 to –5 logarithmic units for ineffective doses (1–100 000 μg/mL or mg/kg bw) and 0 to +8 logarithmic units for effective doses (100 000–0.001 mg/mL or mg/kg bw). A scale illustrating the LDU values is shown in Figure 1. Negative responses at doses less than 1 mg/mL (mg/kg bw) are set equal to 1. Effectively, an LED value ≥ 100 000 or an HID value ≤ 1 produces an LDU = 0; no quantitative information is gained from such extreme values. The dotted lines at the levels of log dose units 1 and –1 define a 'zone of uncertainty' in which positive results are reported at such high doses (between 10 000 and 100 000 mg/mL or mg/kg bw) or negative results are reported at such low doses (1 to 10 mg/ml or mg/kg bw) as to call into question the adequacy of the test.

Figure 1. Scale of log dose units used on the y-axis of activity profiles

Positive units (μg/mL or mg/kg bw)		Log dose	
0.001		8	----
0.01		7	--
0.1		6	--
1.0		5	--
10		4	--
100		3	--
1000		2	--
10 000		1	--
100 000	1	0	----
	10	–1	--
	100	–2	--
	1000	–3	--
	10 000	–4	--
	100 000	–5	----
	Negative (mg/mL or mg/kg bw)		

In practice, an activity profile is computer generated. A data entry programme is used to store abstracted data from published reports. A sequential file (in ASCII) is created for each compound, and a record within that file consists of the name and Chemical Abstracts Service number of the compound, a three-letter code for the test system (see below), the qualitative test result (with and without an exogenous metabolic system), dose (LED or HID), citation number and additional source information. An abbreviated citation for each publication is stored in a segment of a record accessing both the test data file and the citation file. During processing of the data file, an average of the logarithmic values of the data sub-set is calculated, and the length of the profile line represents this average value. All dose values are plotted for each profile line, regardless of whether results are positive or negative. Results obtained in the absence of an exogenous metabolic system are indicated by a bar (–), and results obtained in the presence of an exogenous metabolic system are indicated by an upward-directed arrow (↑). When all results for a given assay are either positive or negative, the mean of the LDU values is plotted as a solid line; when conflicting data are reported for the same assay (i.e. both positive and negative results), the majority data are shown by a solid line and the minority data by a dashed line (drawn to the extreme conflicting response). In the few cases in which the numbers of positive and negative results are equal, the solid line is drawn in the positive direction and the maximal negative response is indicated with a dashed line. Profile lines are identified by three-letter code words representing the commonly used tests. Code words for most of the test systems in current use in genetic toxicology were defined for the US Environmental Protection Agency's GENE-TOX Program (Waters, 1979; Waters & Auletta, 1981). For *IARC Monographs* Supplement 6, Volume 44 and subsequent volumes, as well as the present series, codes were redefined in a manner that should facilitate inclusion of additional tests. Naming conventions are described below.

Data listings are presented in the text and include end-point and test codes, a short test code definition, results, either with (M) or without (NM) an exogenous activation system, the associated LED or HID value and a short citation. Test codes are organized phylogenetically and by end-point from left to right across each activity profile and from top to bottom of the corresponding data listing. End-points are defined as follows: A, aneuploidy; C, chromosomal aberrations; D, DNA damage; F, assays of body fluids; G, gene mutation; H, host-mediated assays; I, inhibition of intercellular communication; M, micronuclei; P, sperm morphology; R, mitotic recombination or gene conversion; S, sister chromatid exchange; and T, cell transformation.

Dose conversions for activity profiles

Doses are converted to mg/mL for in-vitro tests and to mg/kg bw per day for in-vivo experiments.

1. In-vitro test systems

(*a*) Weight/volume converts directly to mg/mL.

(*b*) Molar (M) concentration × molecular weight = mg/mL = 103 mg/mL; mM concentration × molecular weight = mg/mL.

(*c*) Soluble solids expressed as % concentration are assumed to be in units of mass per volume (i.e. 1% = 0.01 g/mL = 10 000 µg/mL; also, 1 ppm = 1 mg/mL).

(*d*) Liquids and gases expressed as % concentration are assumed to be given in units of volume per volume. Liquids are converted to weight per volume using the density (D) of the solution (D = g/mL). Gases are converted from volume to mass using the ideal gas law, $PV = nRT$. For exposure at 20–37 °C at standard atmospheric pressure, 1% (v/v) = 0.4 mg/mL × molecular weight of the gas. Also, 1 ppm (v/v) = 4×10^5 mg/mL × molecular weight.

(*e*) In microbial plate tests, it is usual for the doses to be reported as weight/plate, whereas concentrations are required to enter data on the activity profile chart. While remaining cognisant of the errors involved in the process, it is assumed that a 2-mL volume of top agar is delivered to each plate and that the test substance remains in solution within it; concentrations are derived from the reported weight/plate values by dividing by this arbitrary volume. For spot tests, a 1-mL volume is used in the calculation.

(f) Conversion of particulate concentrations given in mg/cm^2 is based on the area (*A*) of the dish and the volume of medium per dish; i.e. for a 100-mm dish: $A = \pi R^2 = \pi \times (5 \text{ cm})^2 = 78.5 \text{ cm}^2$. If the volume of medium is 10 mL, then 78.5 cm^2 = 10 mL and 1 cm^2 = 0.13 mL.

2. In-vitro systems using in-vivo activation

For the body fluid-urine (BF-) test, the concentration used is the dose (in mg/kg bw) of the compound administered to test animals or patients.

3. In-vivo test systems

(*a*) Doses are converted to mg/kg bw per day of exposure, assuming 100% absorption. Standard values are used for each sex and species of rodent, including body weight and average intake per day, as reported by Gold *et al.* (1984). For example, in a test using male mice fed 50 ppm of the agent in the diet, the standard food intake per day is 12% of body weight, and the conversion is dose = 50 ppm × 12% = 6 mg/kg bw per day. Standard values used for humans are: weight—males, 70 kg; females, 55 kg; surface area, 1.7 m^2; inhalation rate, 20 L/min for light work, 30 L/min for mild exercise.

(*b*) When reported, the dose at the target site is used. For example, doses given in studies of lymphocytes of humans exposed *in vivo* are the measured blood concentrations in mg/mL.

Codes for test systems

For specific nonmammalian test systems, the first two letters of the three-symbol code word define the test organism (e.g. SA- for *Salmonella typhimurium*, EC- for *Escherichia coli*). If the species is not known, the convention used is -S-. The third symbol may be used to define the tester strain (e.g. SA8 for *S. typhimurium* TA1538, ECW for *E. coli* WP2uvrA). When strain designation is not indicated, the third letter is used to define the specific genetic end-point under investigation (e.g. --D for differential toxicity, --F for forward mutation, --G for gene conversion or genetic crossing-over, --N for aneuploidy, --R for reverse mutation, --U for unscheduled DNA synthesis). The third letter may also be used to define the general end-point under investigation when a more complete definition is not possible or relevant (e.g. --M for mutation, --C for chromosomal aberration). For mammalian test systems, the first letter of the three-letter code word defines the genetic end-point under investigation: A-- for aneuploidy, B-- for binding, C-- for chromosomal aberration, D-- for DNA strand breaks, G-- for gene mutation, I-- for inhibition of intercellular communication, M-- for micronucleus formation, R-- for DNA repair, S-- for sister chromatid exchange, T-- for cell transformation and U-- for unscheduled DNA synthesis.

For animal (i.e. non-human) test systems *in vitro*, when the cell type is not specified, the code letters -IA are used. For such assays *in vivo*, when the animal species is not specified, the code letters -VA are used. Commonly used animal species are identified by the third letter (e.g. --C for Chinese hamster, --M for mouse, --R for rat, --S for Syrian hamster).

For test systems using human cells *in vitro*, when the cell type is not specified, the code letters -IH are used. For assays on humans *in vivo*, when the cell type is not specified, the code letters -VH are used. Otherwise, the second letter specifies the cell type under investigation (e.g. -BH for bone marrow, -LH for lymphocytes).

Some other specific coding conventions used for mammalian systems are as follows: BF- for body fluids, HM- for host-mediated, --L for leukocytes or lymphocytes *in vitro* (-AL, animals; -HL, humans), -L- for leukocytes *in vivo* (-LA, animals; -LH, humans),--T for transformed cells.

Note that these are examples of major conventions used to define the assay code words. The alphabetized listing of codes must be examined to confirm a specific code word. As might be expected from the limitation to three symbols, some codes do not fit the naming conventions precisely. In a few cases, test systems are defined by first-letter code words, for example: MST, mouse spot test; SLP, mouse specific locus mutation, postspermatogonia; SLO, mouse specific locus mutation, other stages; DLM, dominant lethal mutation in mice; DLR, dominant lethal mutation in rats; MHT, mouse heritable translocation.

The genetic activity profiles and listings were prepared in collaboration with Environmental Health Research and Testing Inc. (EHRT) under contract to the United States Environmental Protection Agency; EHRT also determined the doses used. The references cited in each genetic activity profile listing can be found in the list of references in the appropriate volume.

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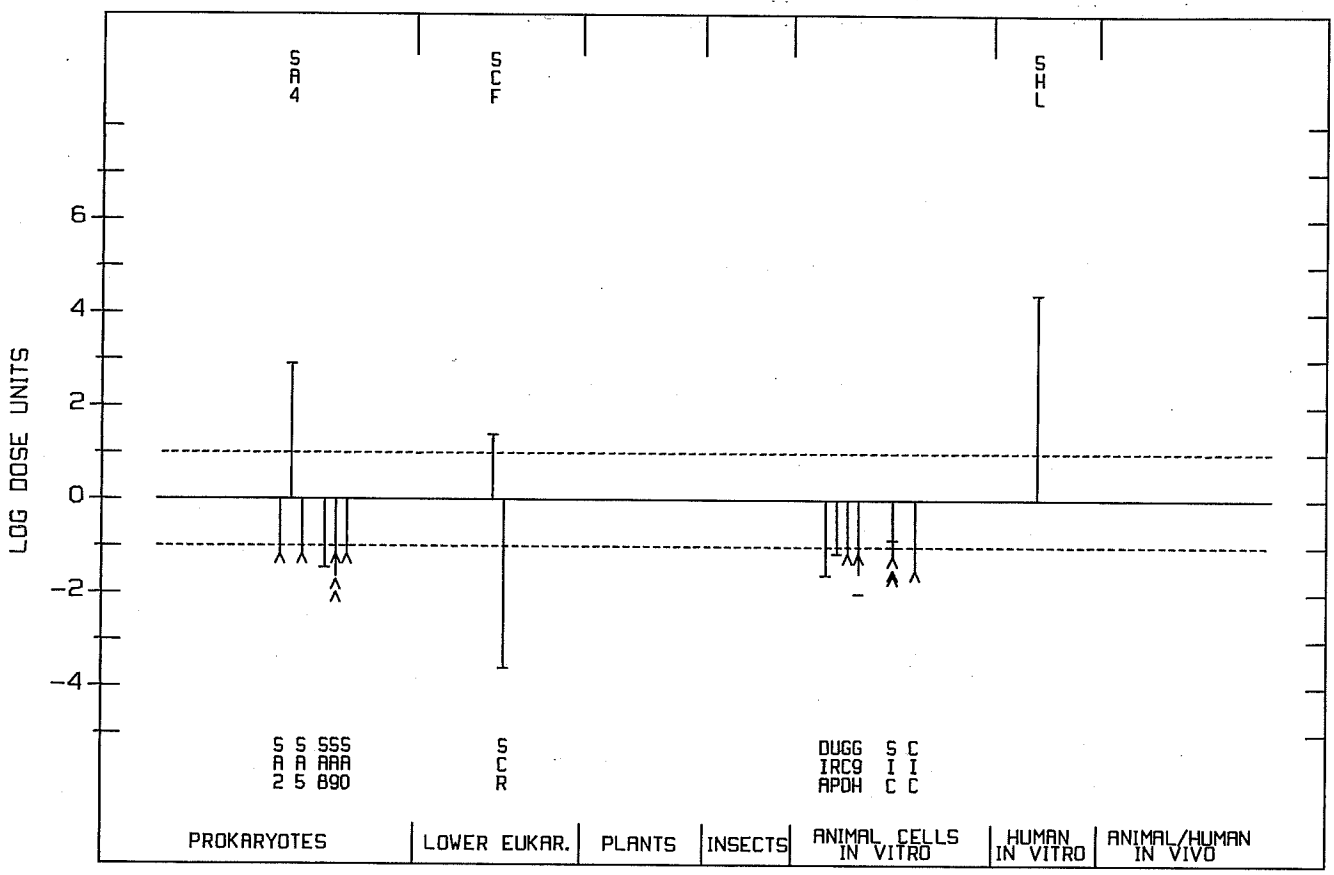
RETINOL



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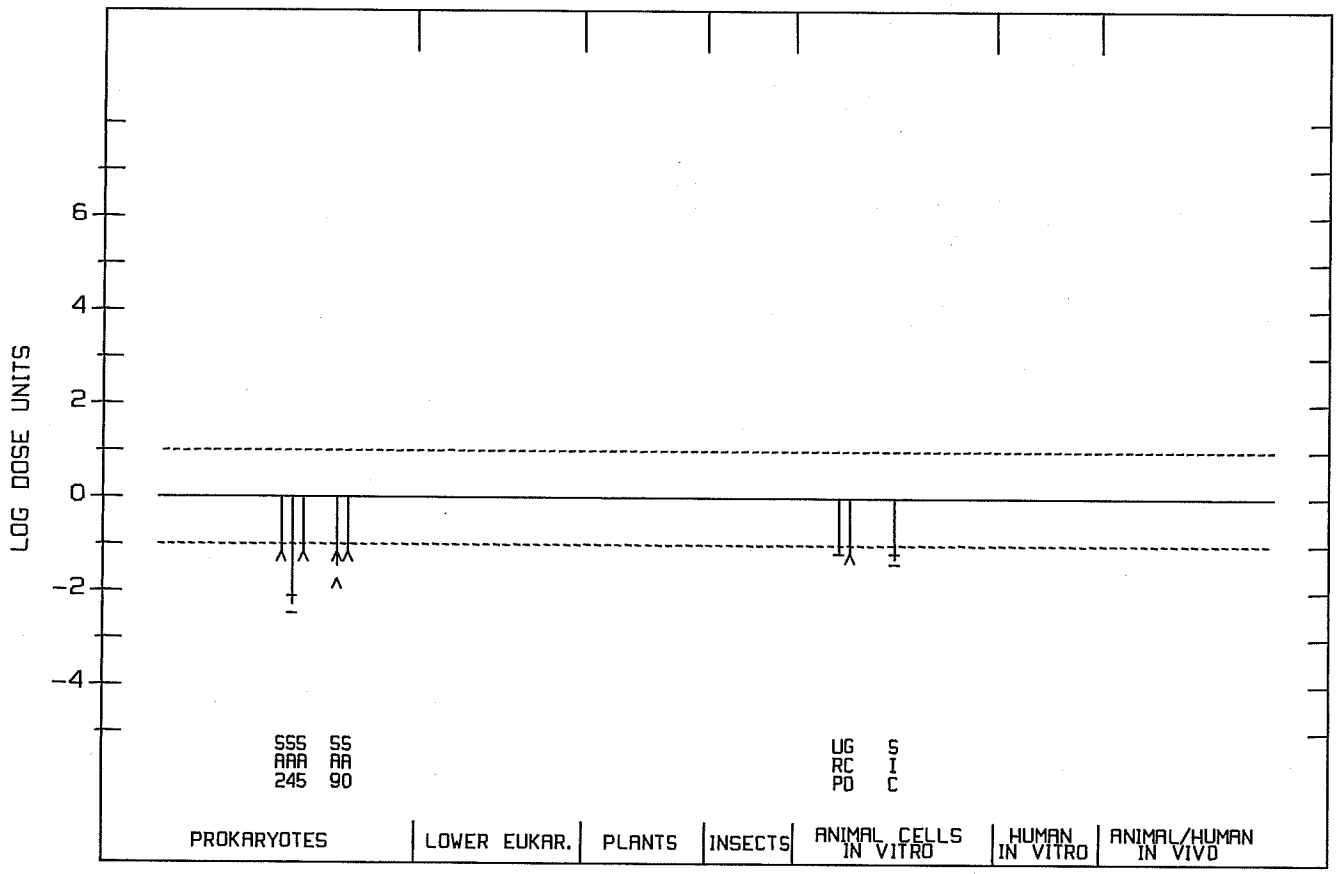

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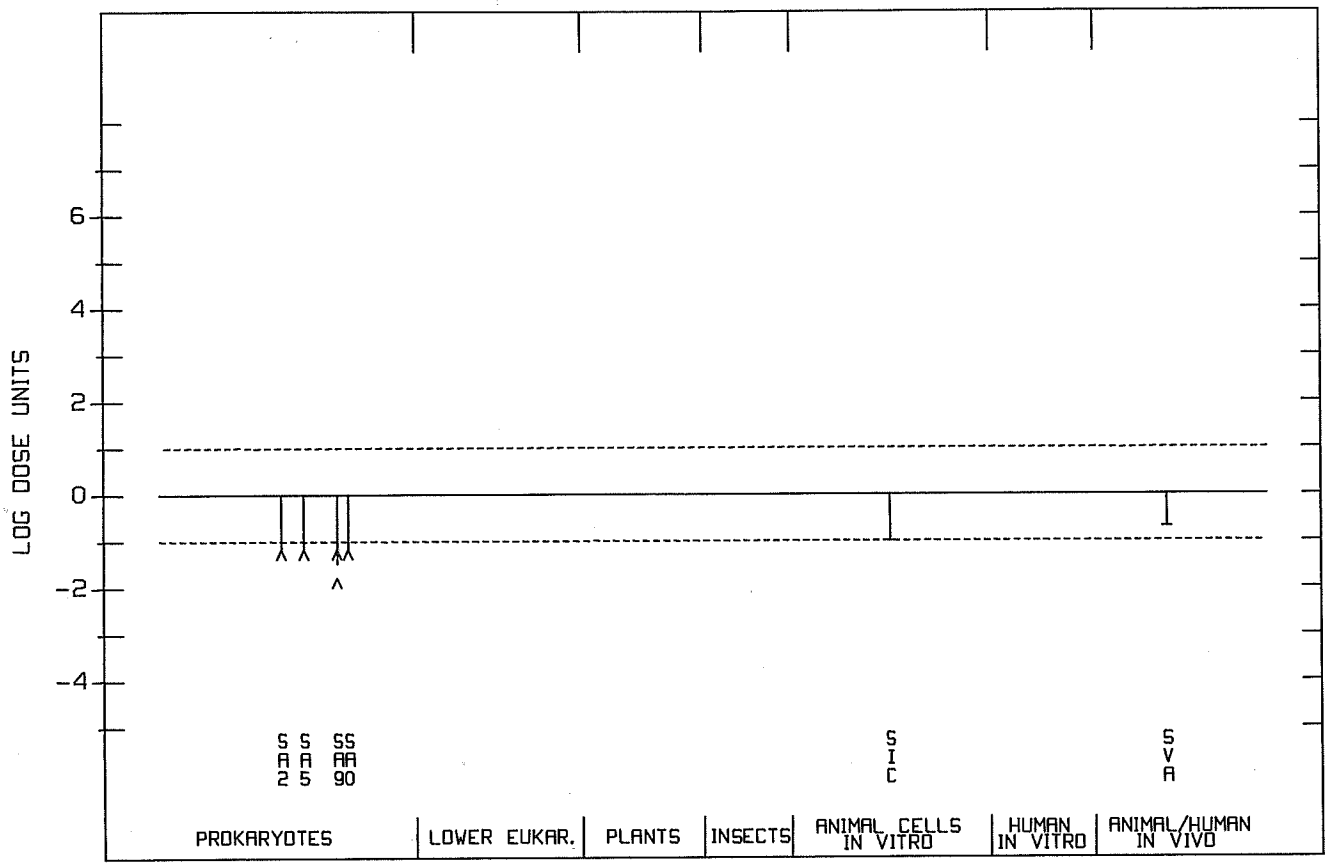

RETINYL PALMITATE