APPENDIX 2

ACTIVITY PROFILES FOR GENETIC AND RELATED EFFECTS

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Methods

The x-axis of the activity profile (Waters *et al.*, 1987, 1988) represents the bioassays in phylogenetic sequence by endpoint, 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 molecular weights 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.

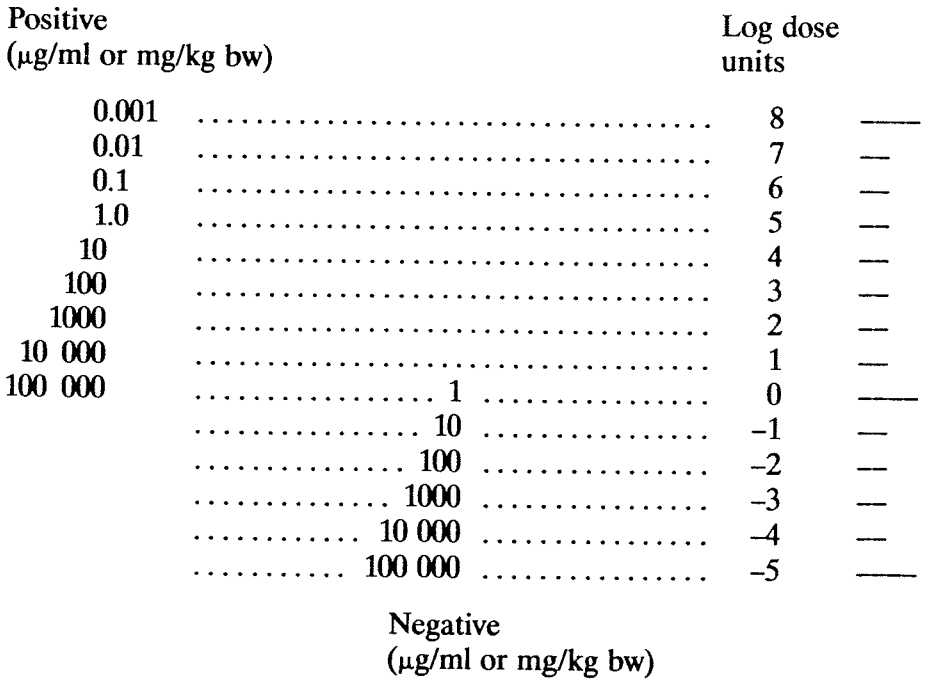
In order to accommodate both the wide range of doses encountered and positive and negative responses on a continuous scale, doses are transformed logarithmically, so that effective (LED) and ineffective (HID) doses are represented by positive and negative

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
$$LDU_{ij} = -\log_{10}(\text{dose} \times 10^{-5}), \text{ for LED values; LDU} \geq 0. \quad (1)$$

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 μg/ml or mg/kg bw). A scale illustrating the LDU values is shown in Figure 1. Negative responses at doses less than 1 μg/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 μg/ml or mg/kg bw) or negative results are reported at such low dose levels (1 to 10 μg/ml or mg/kg bw) as to call into question the adequacy of the test.

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



LED and HID are expressed as μg/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 subset 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, including this publication, 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 endpoint 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 endpoint from left to right across each activity profile and from top to bottom of the corresponding data listing. Endpoints 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 μg/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 μg/ml.
   (b) Molar (M) concentration × molecular weight = mg/ml = 10^3 μg/ml; mM concentration × molecular weight = μg/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 μg/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 μg/ml × molecular weight of the gas. Also, 1 ppm (v/v) = 4×10^{-5} μg/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 $\mu g/cm^2$ are based on the area (A) of the dish and the volume of medium per dish; i.e., for a 100-mm dish: $A = \pi R^2 = \pi \times (5\ cm)^2 = 78.5\ cm^2$. 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 μg/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* WP2*uvrA*). When strain designation is not indicated, the third letter is used to define the specific genetic endpoint 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 endpoint 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 endpoint 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 test, postspermatogonia; SLO, mouse specific locus test, other stages; DLM, dominant lethal test in mice; DLR, dominant lethal test in rats; MHT, mouse heritable translocation test.

The genetic activity profiles and listings were prepared in collaboration with Environmental Health Research and Testing Inc. (EHRT) under contract to the US 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 monograph.

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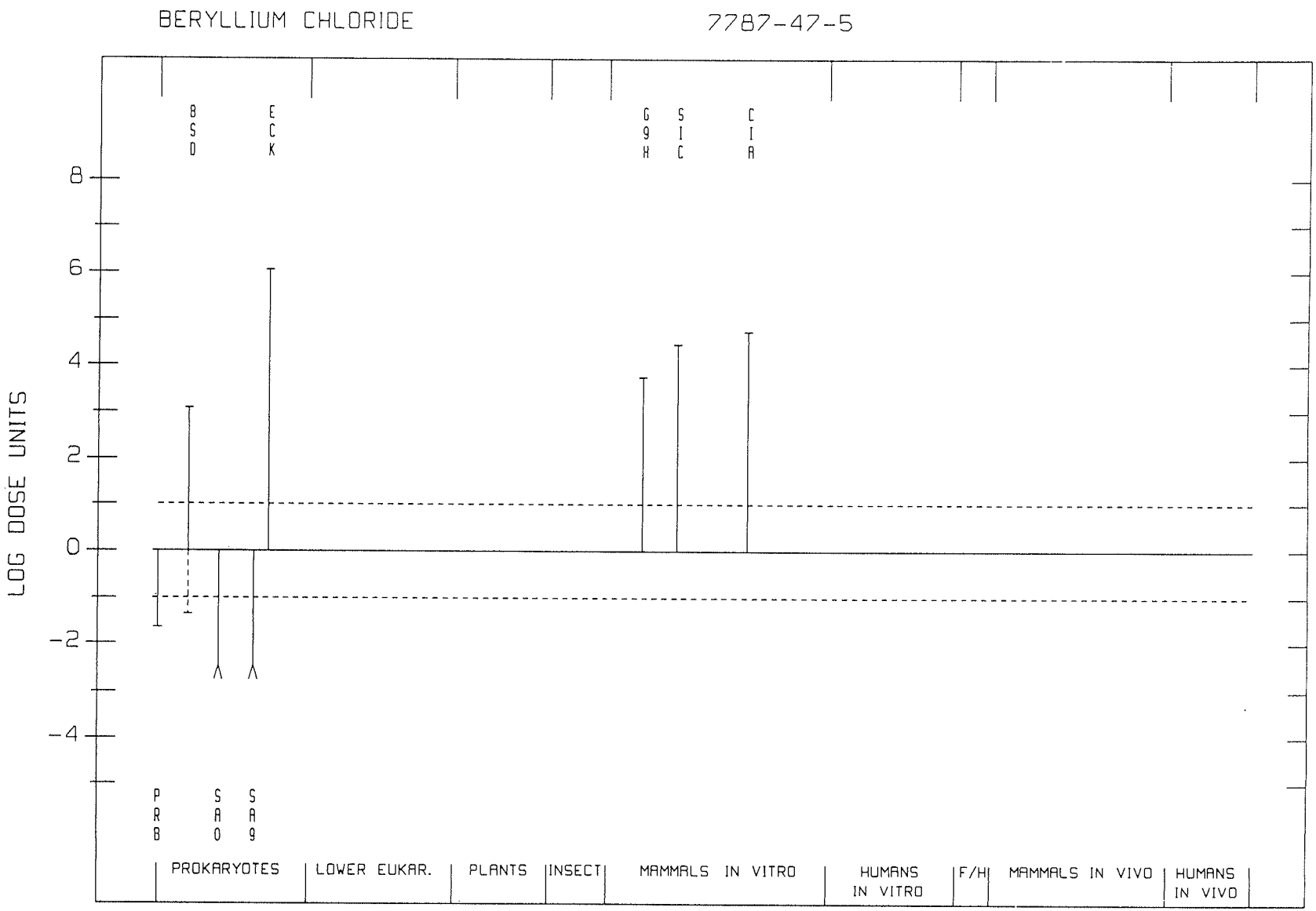
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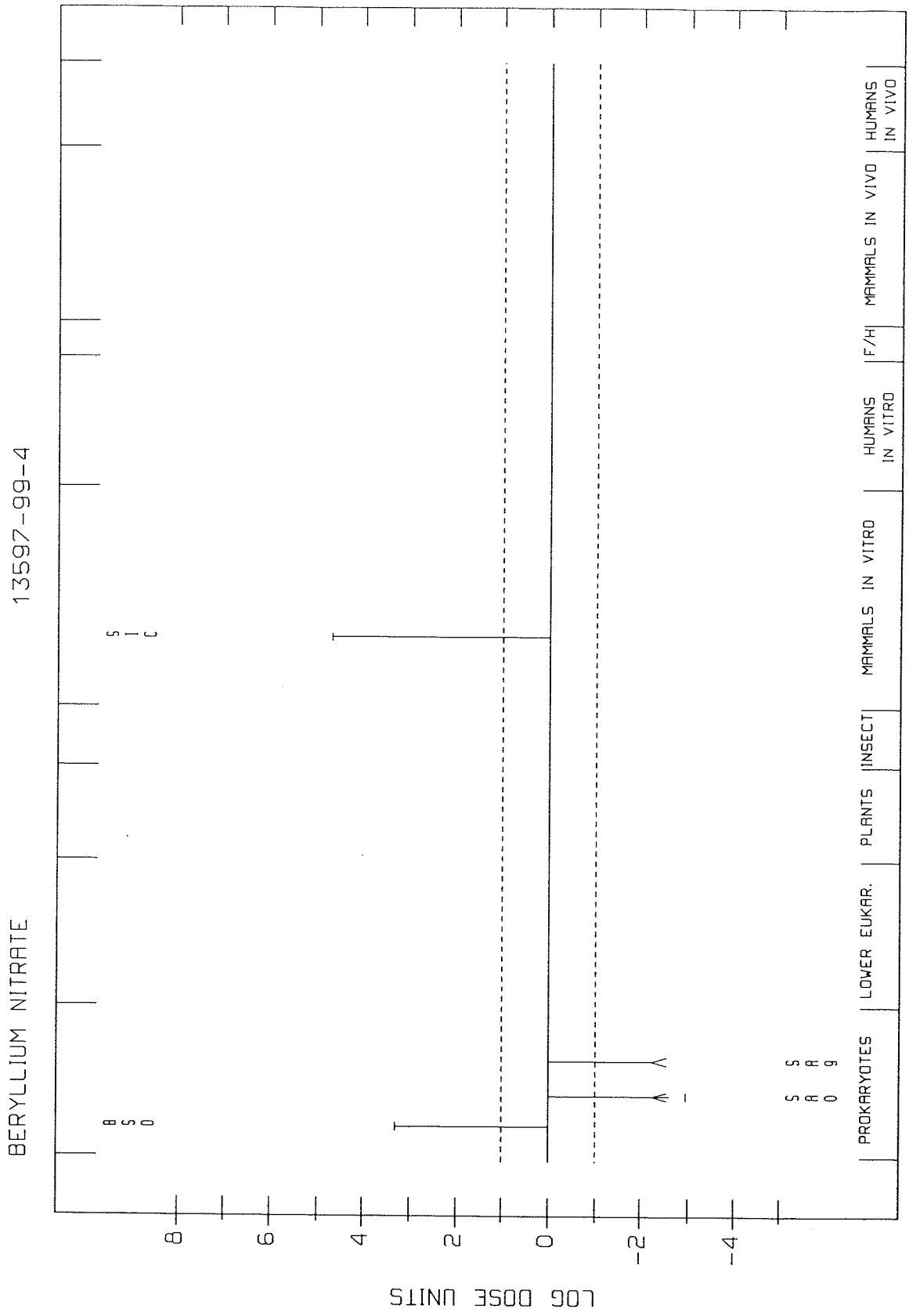
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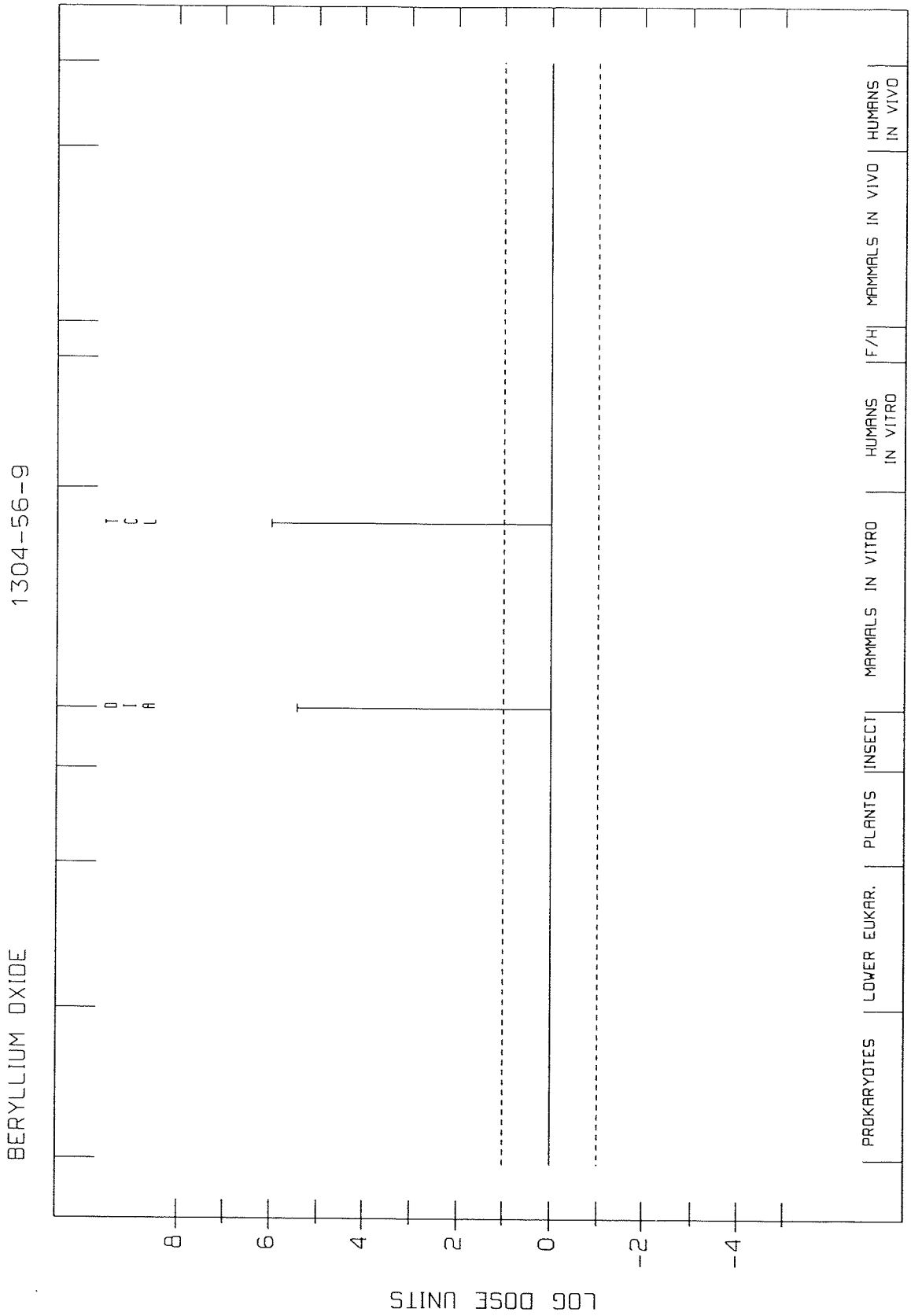
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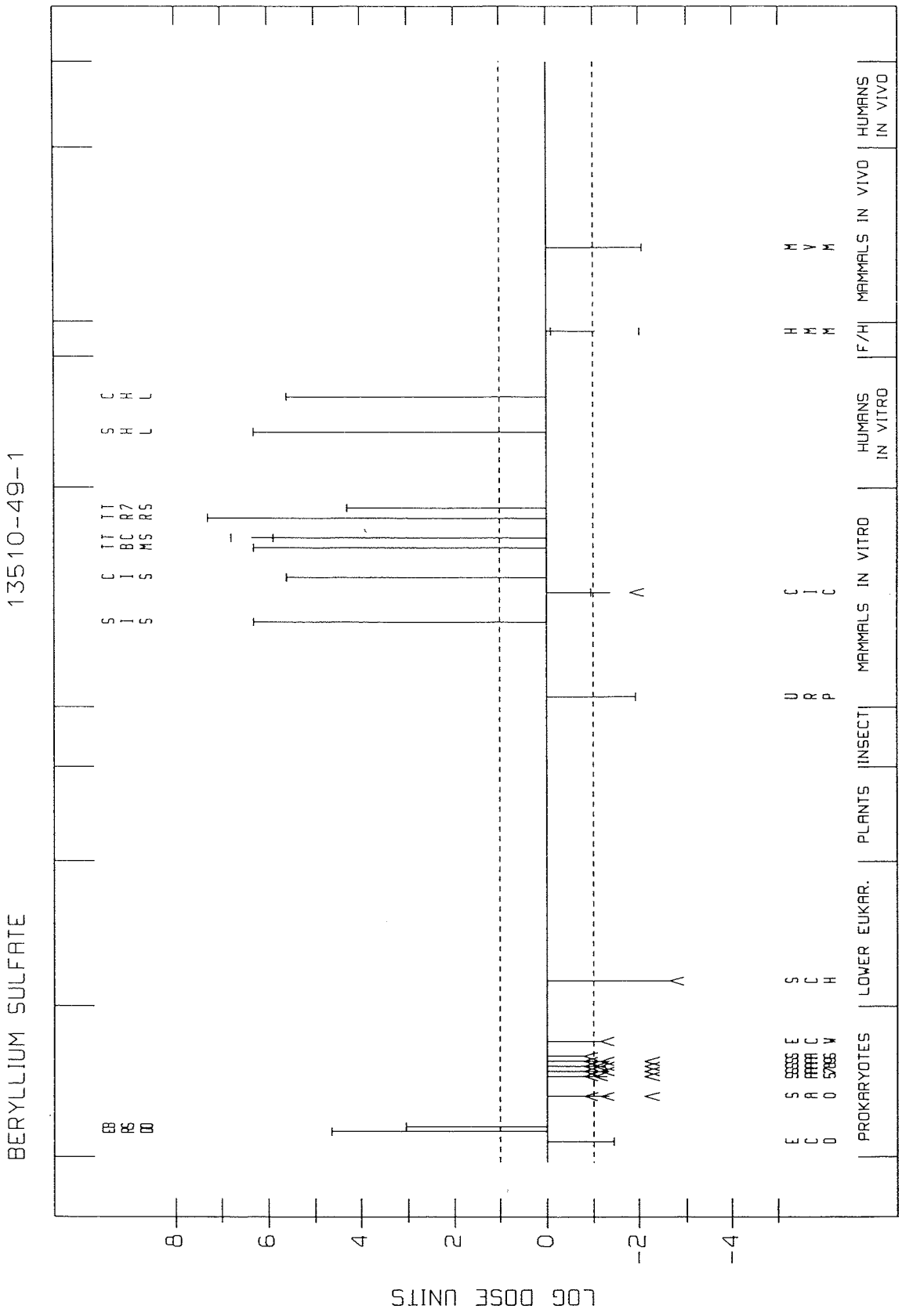
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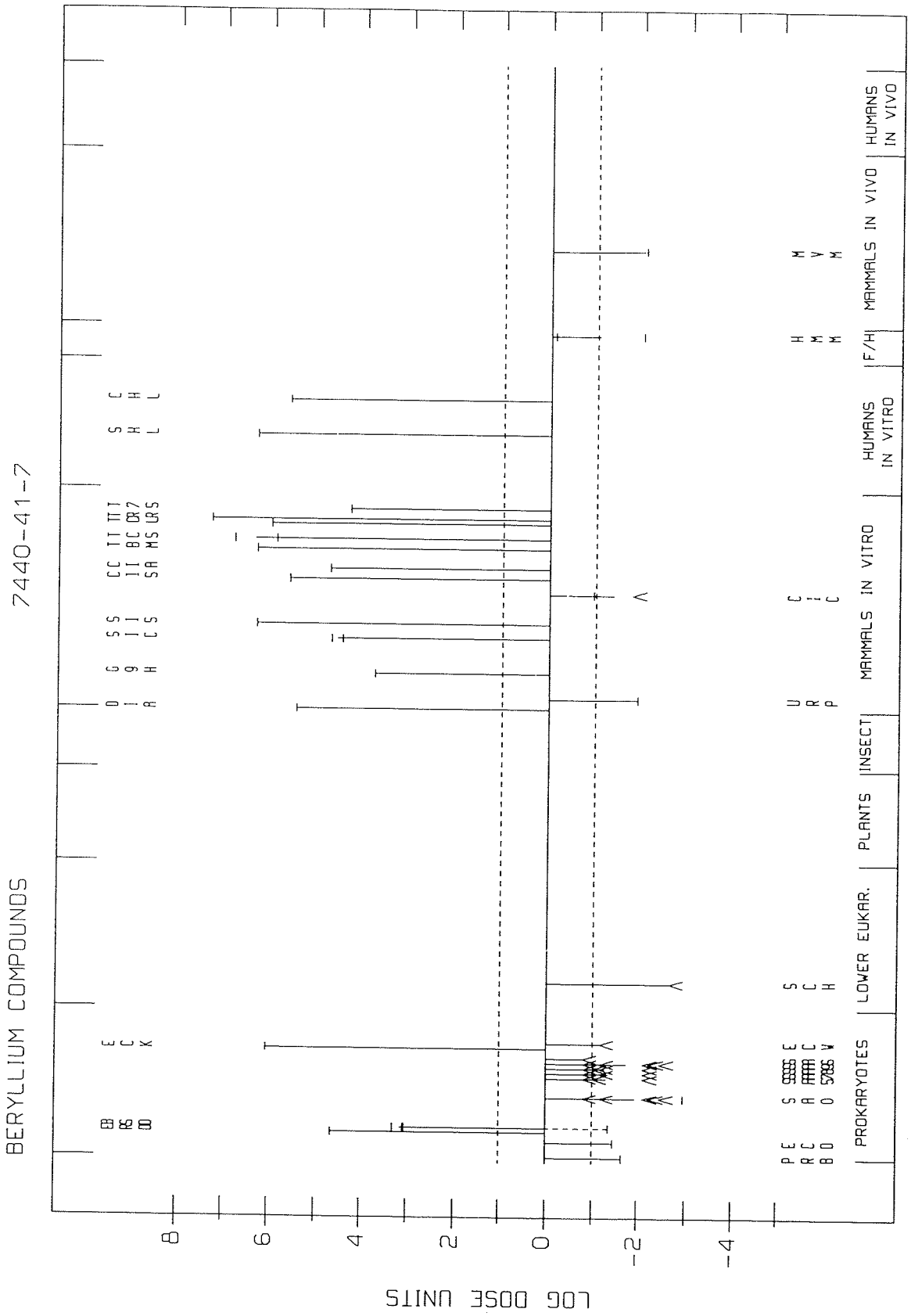
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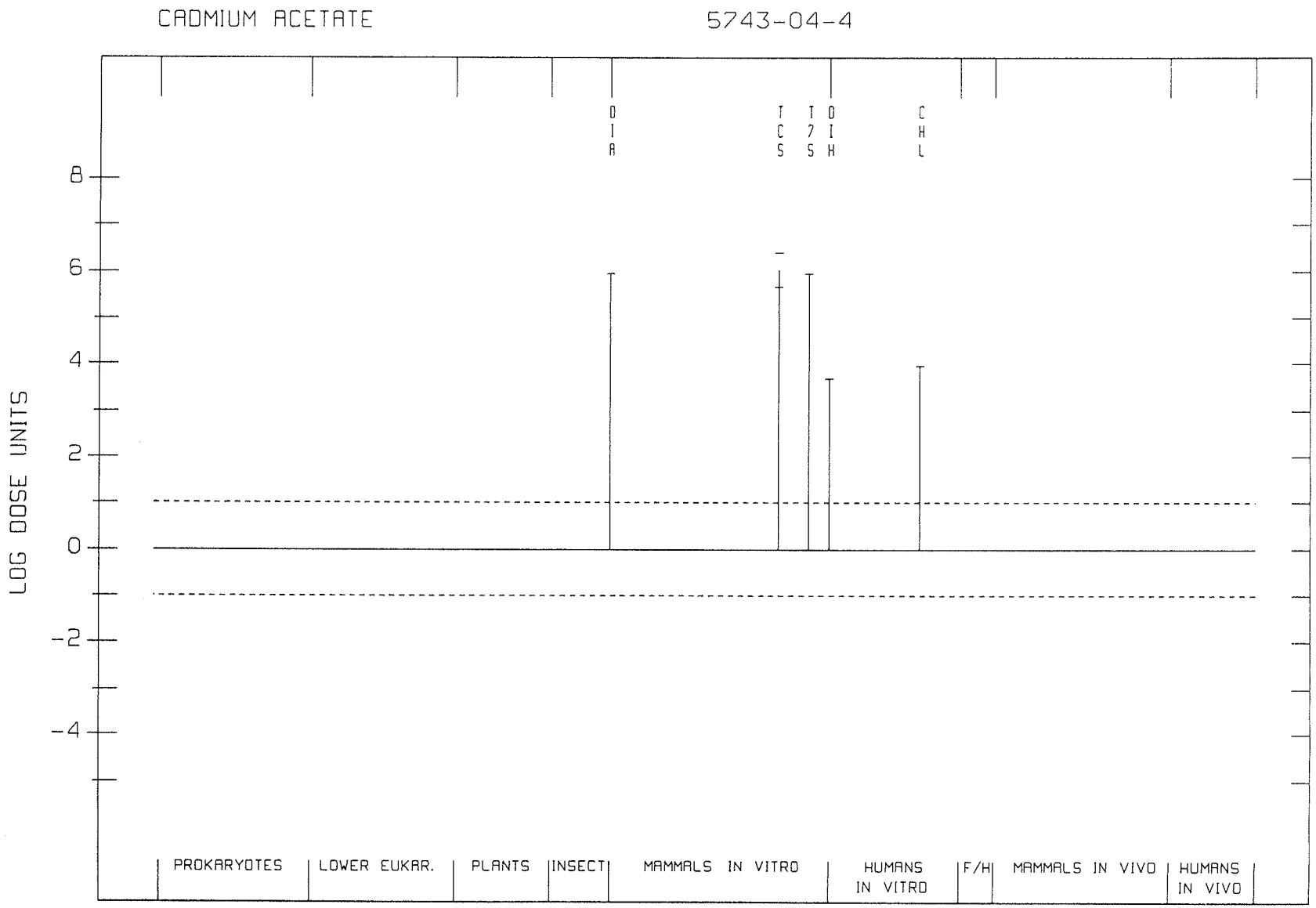
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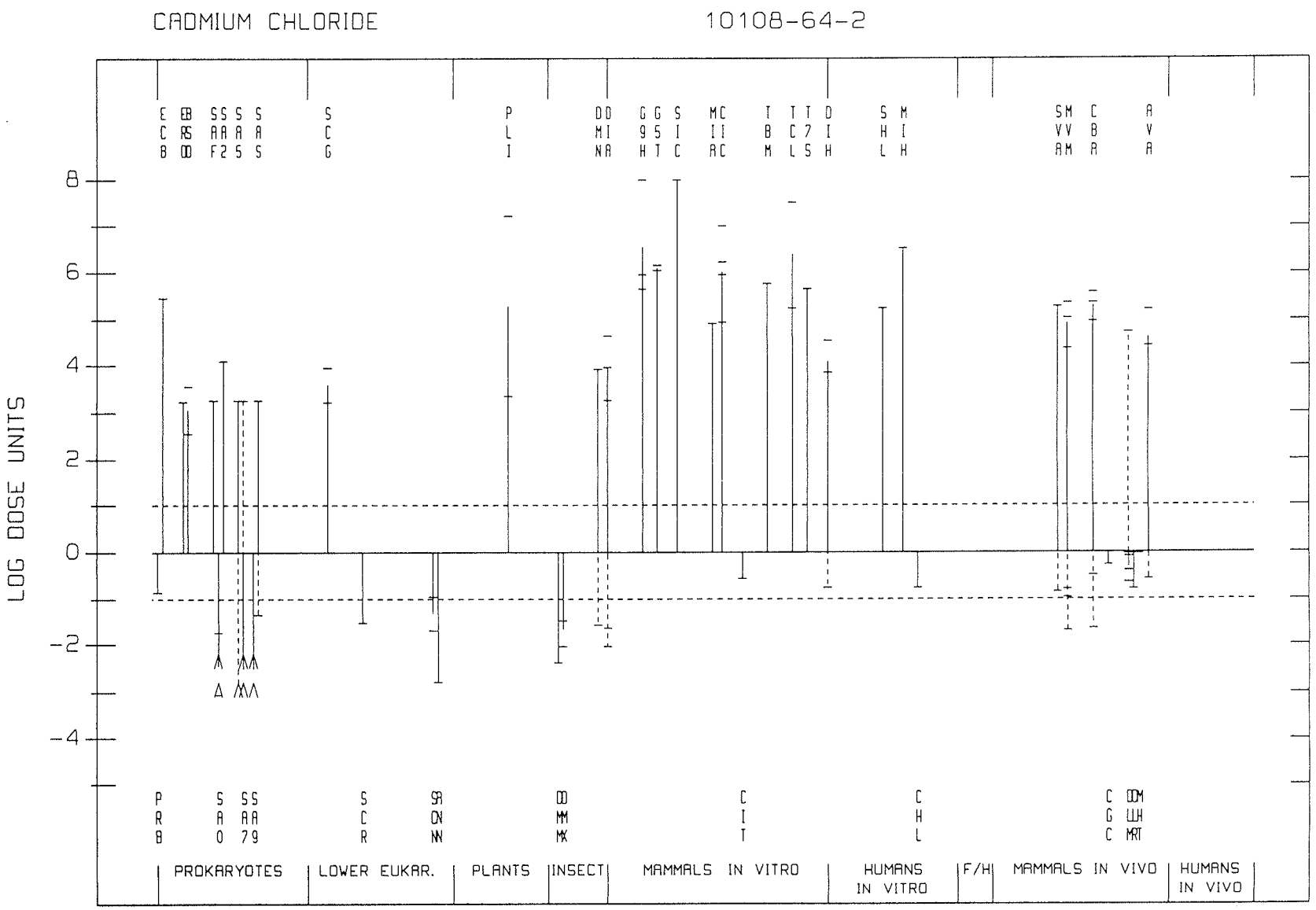
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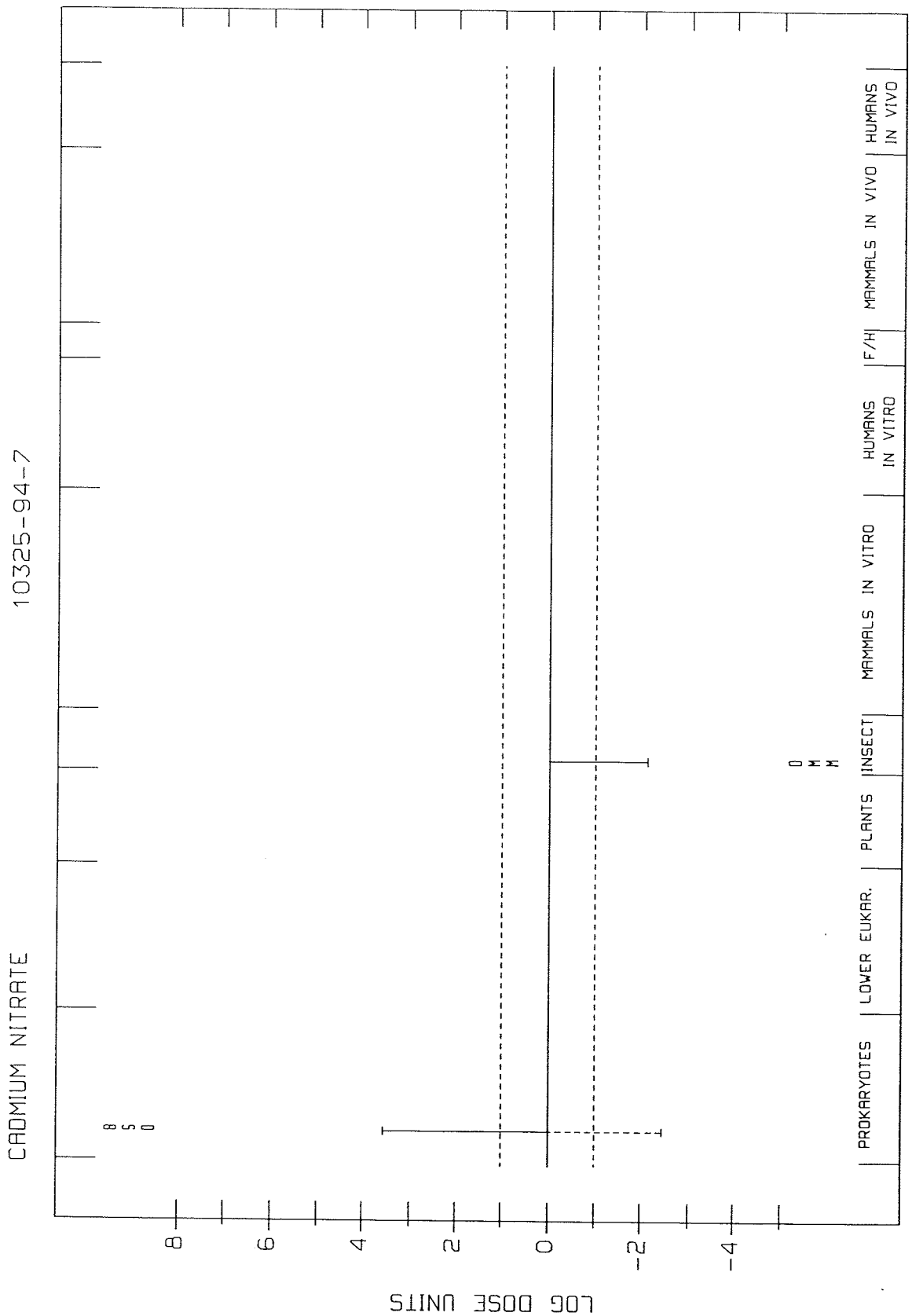
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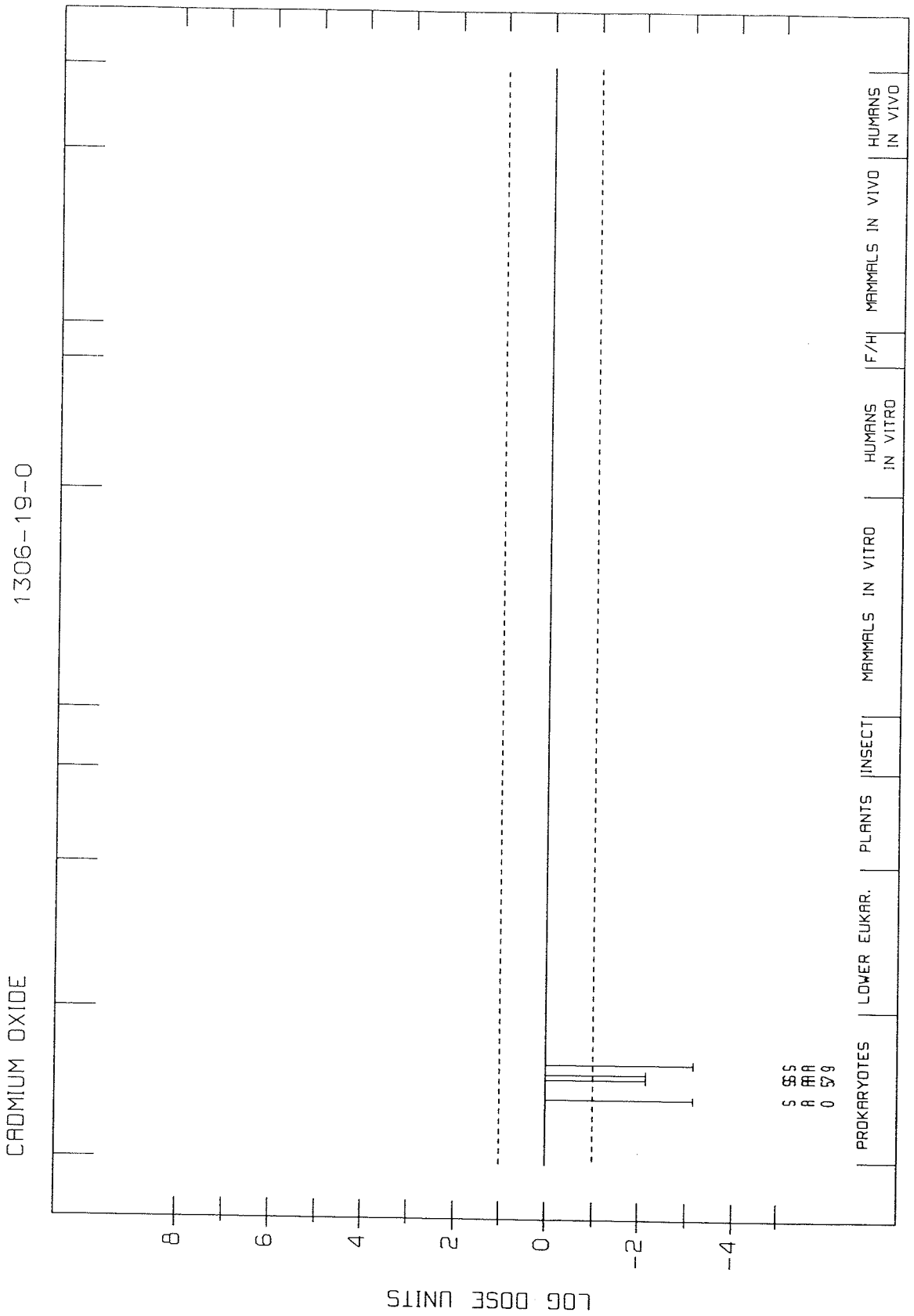
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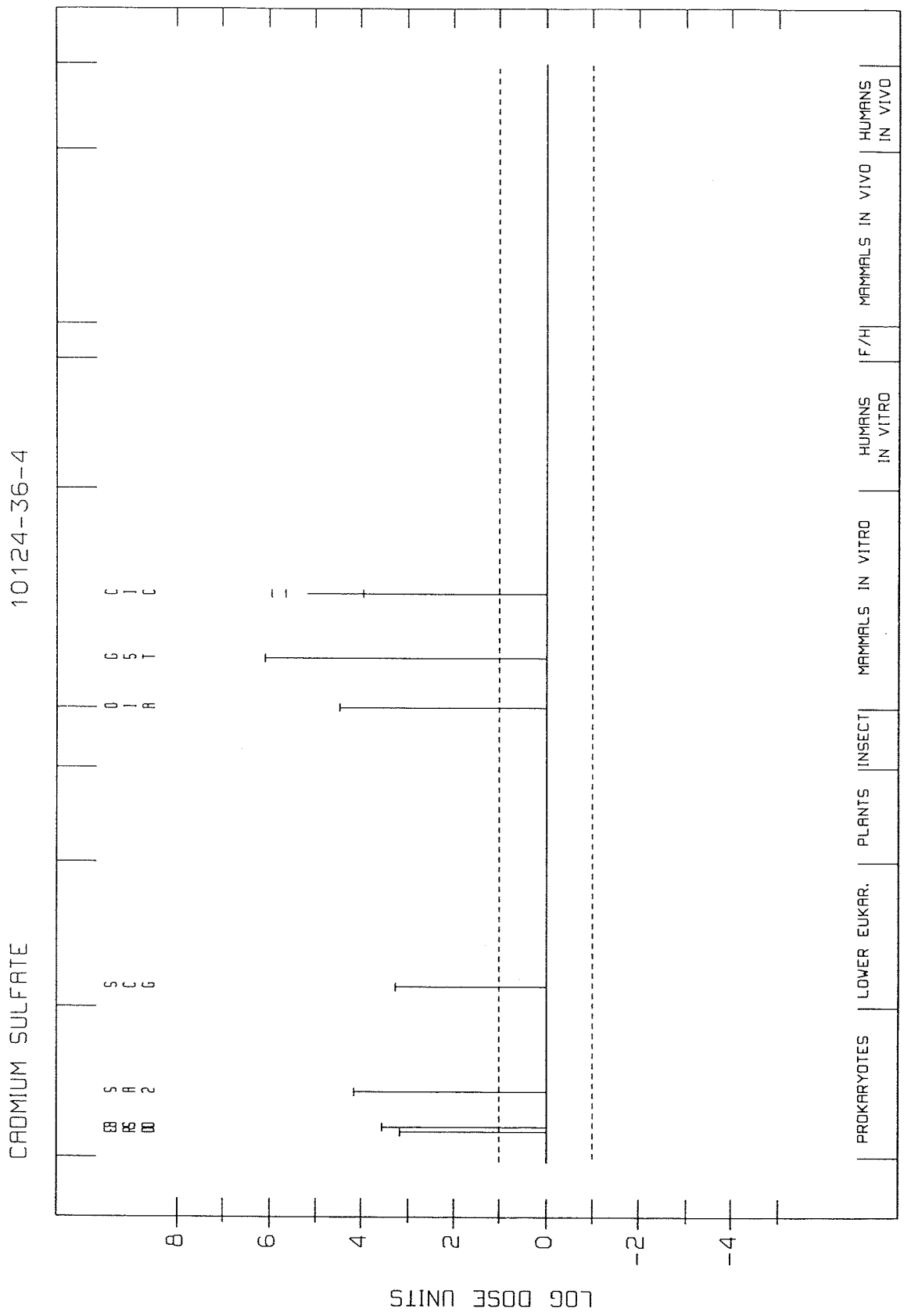
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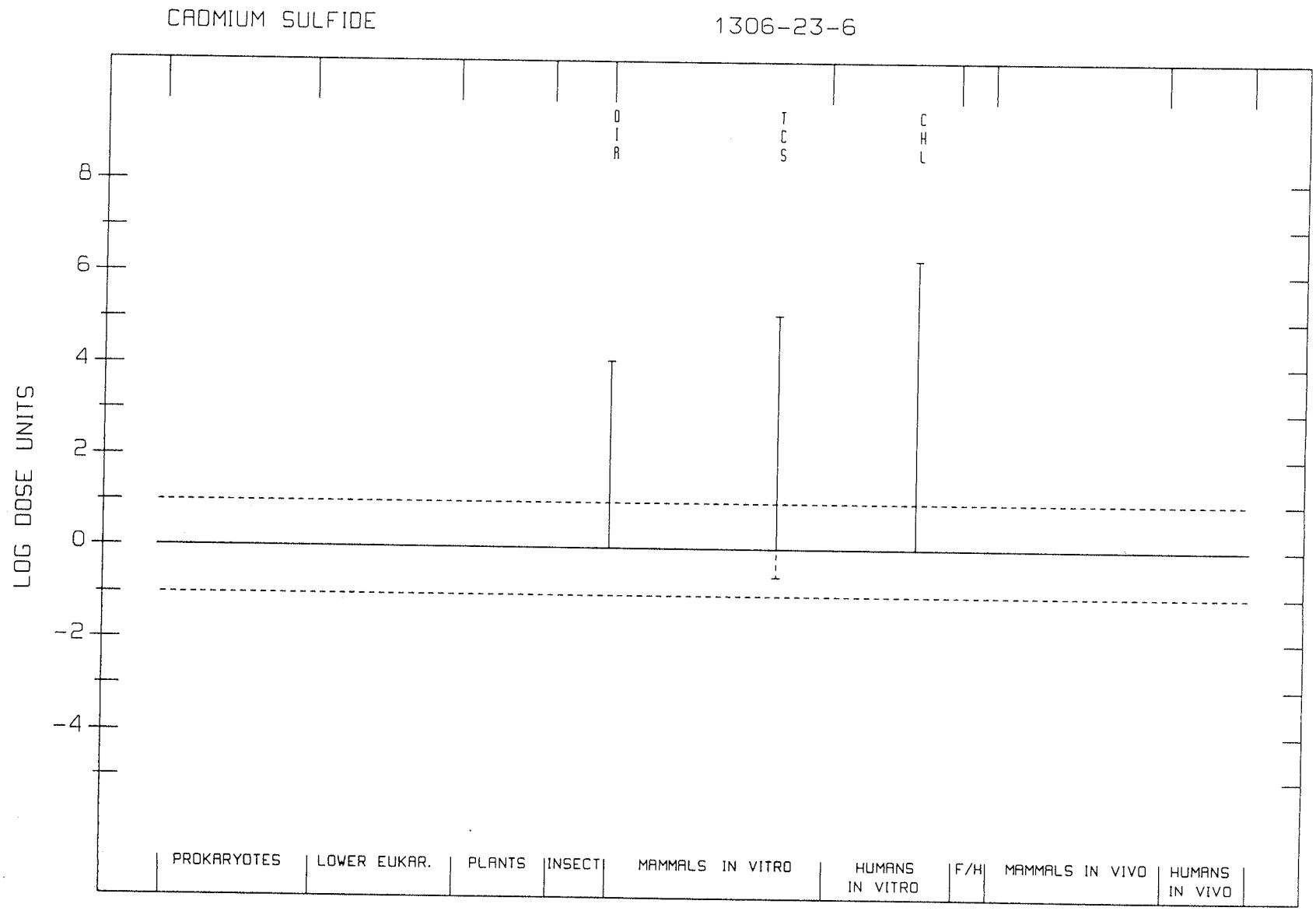
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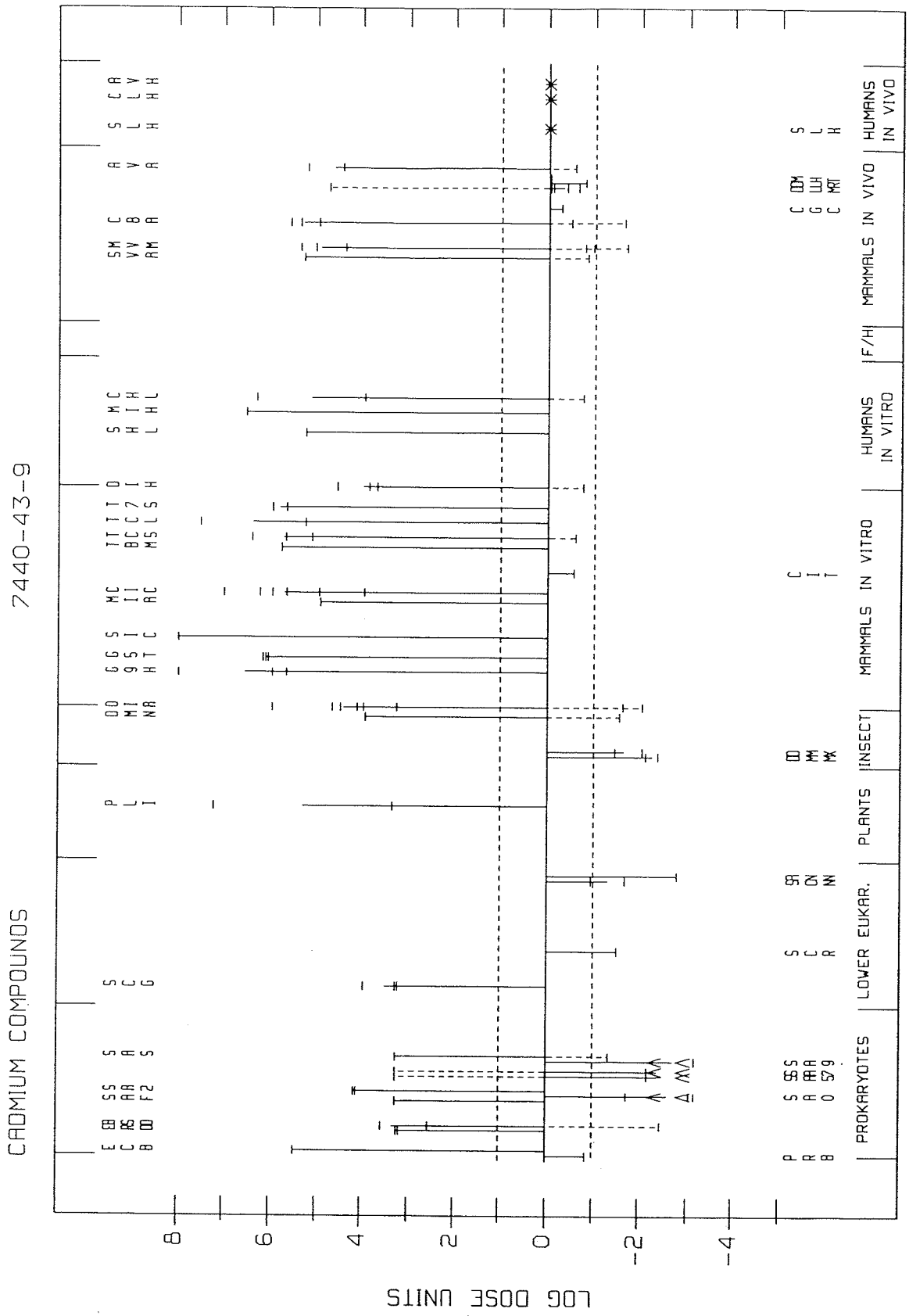
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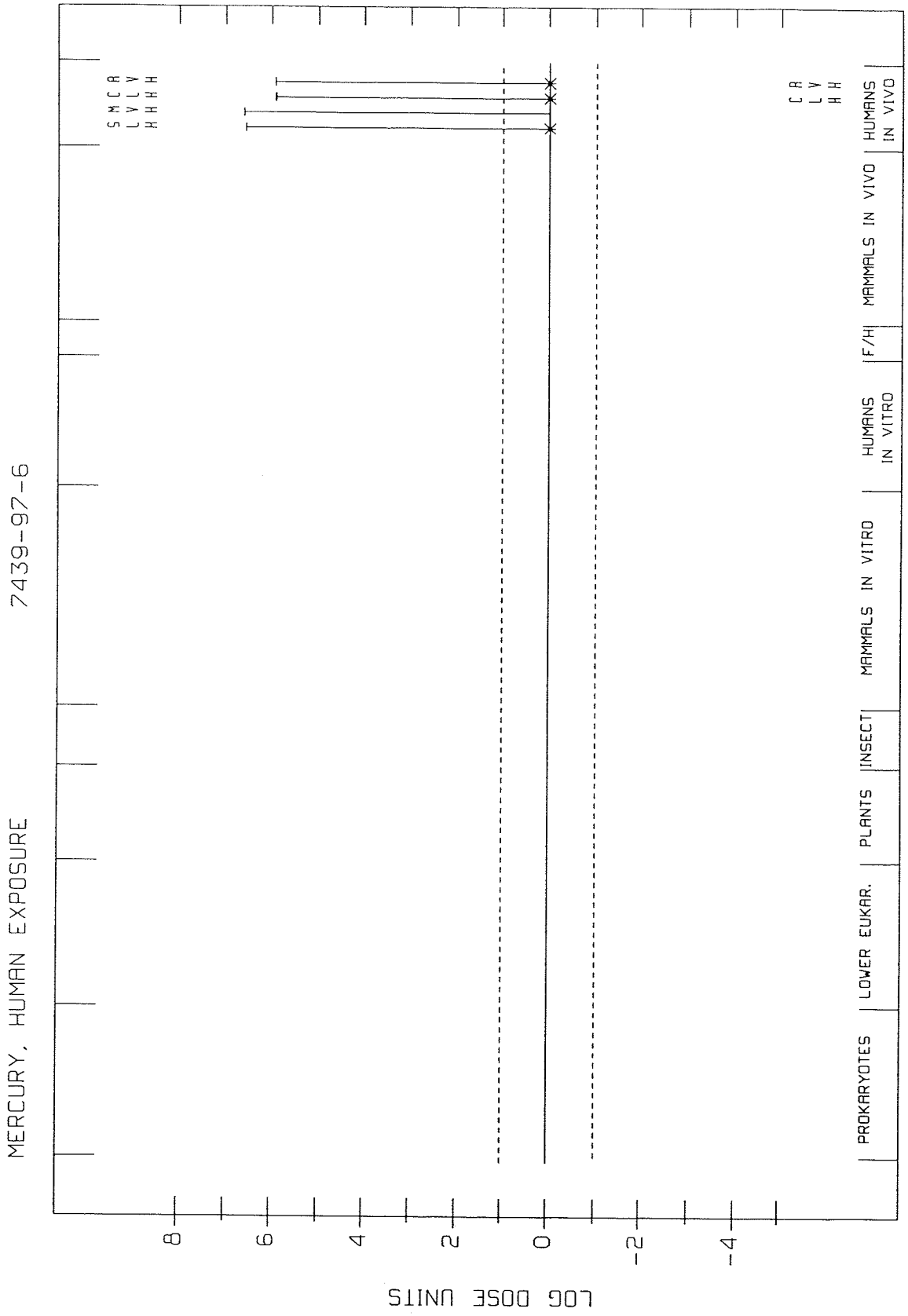
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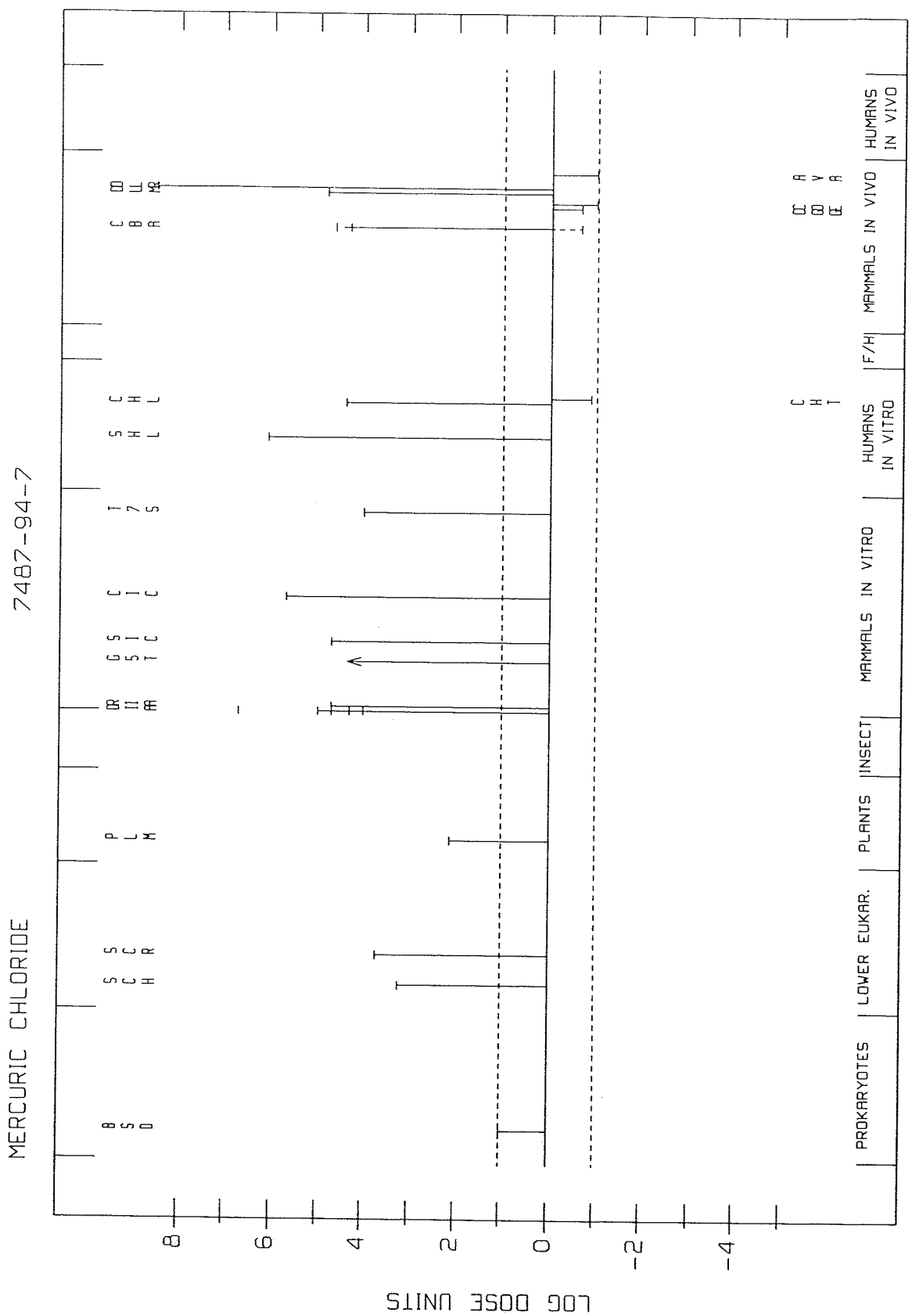
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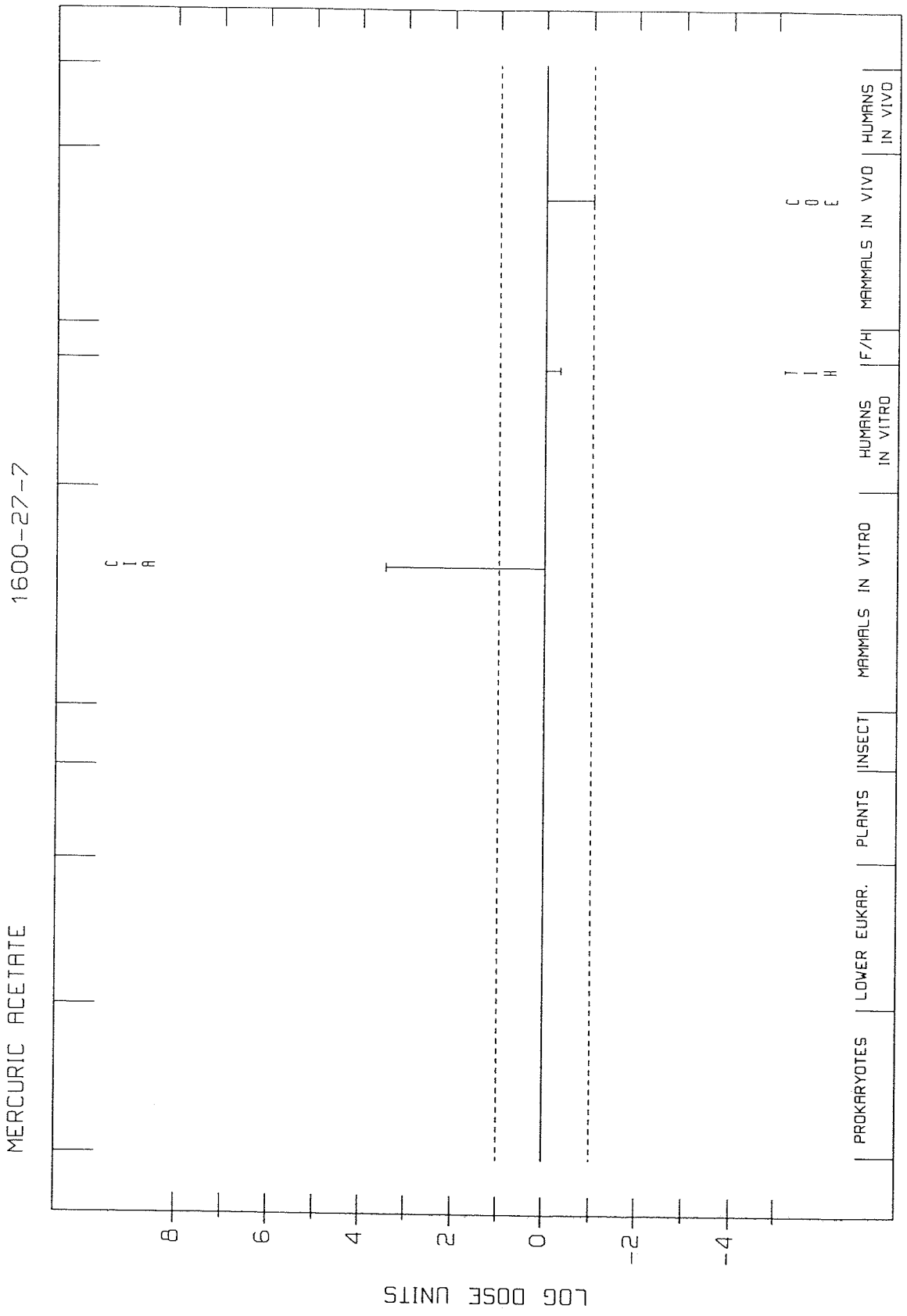
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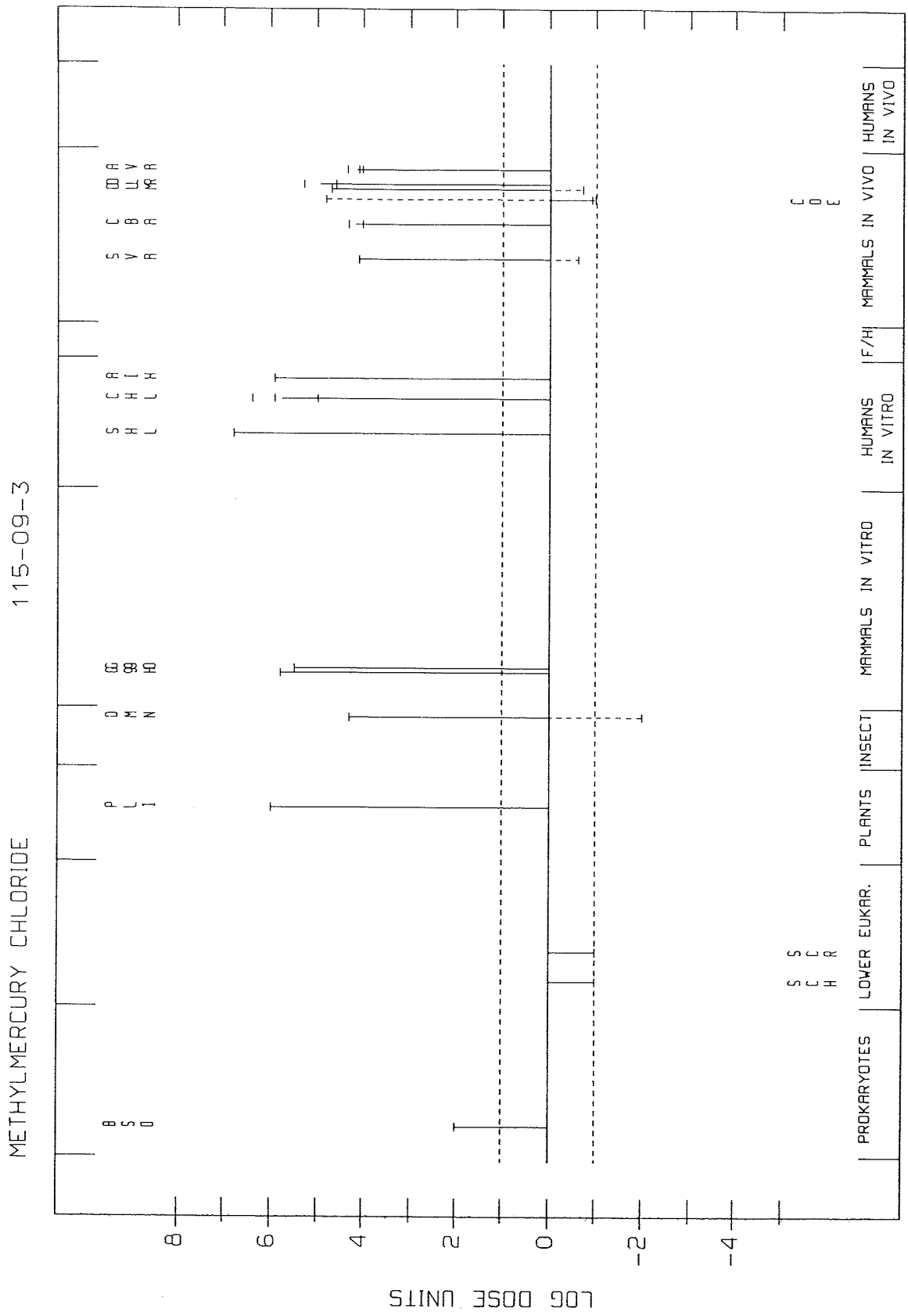
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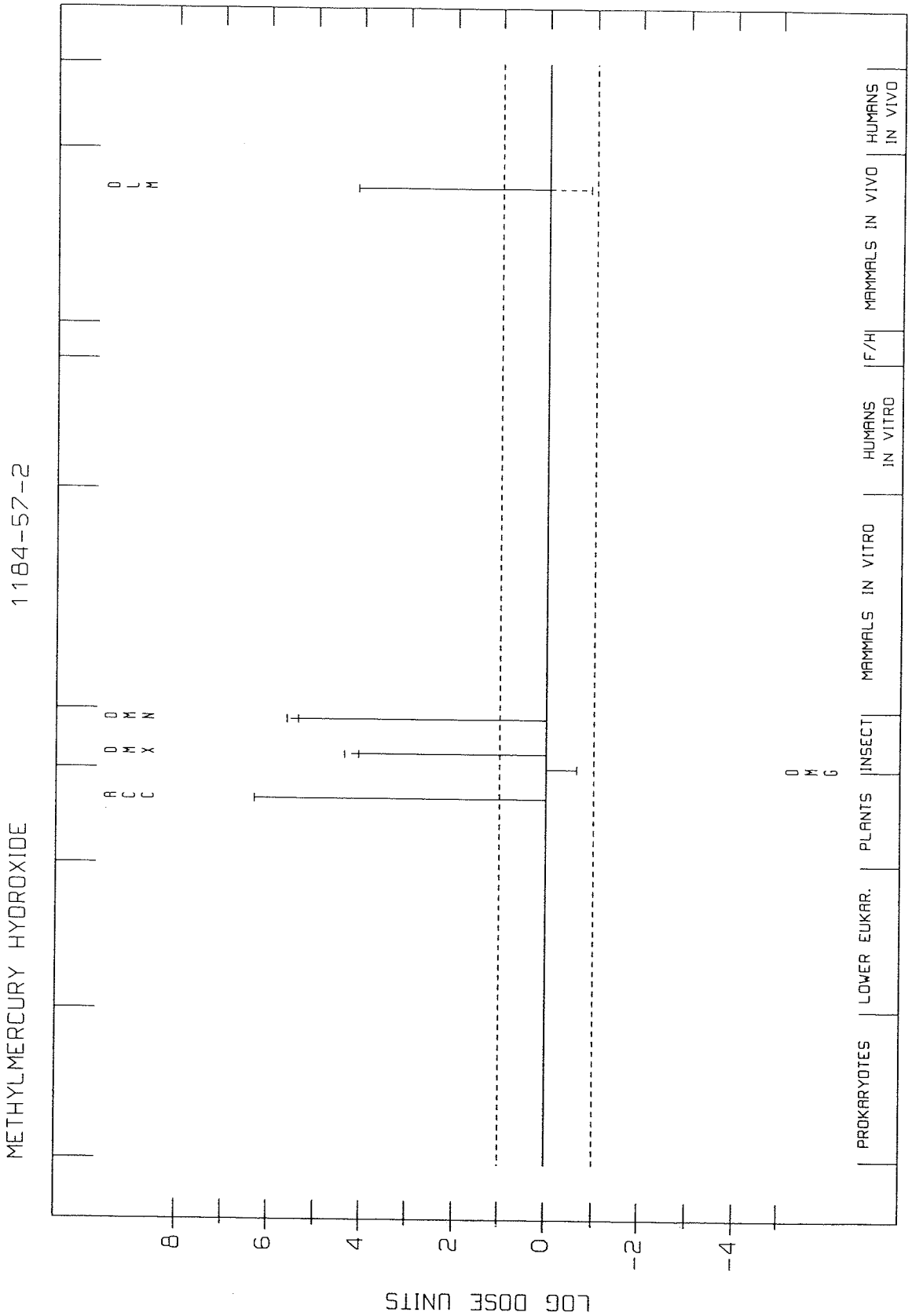
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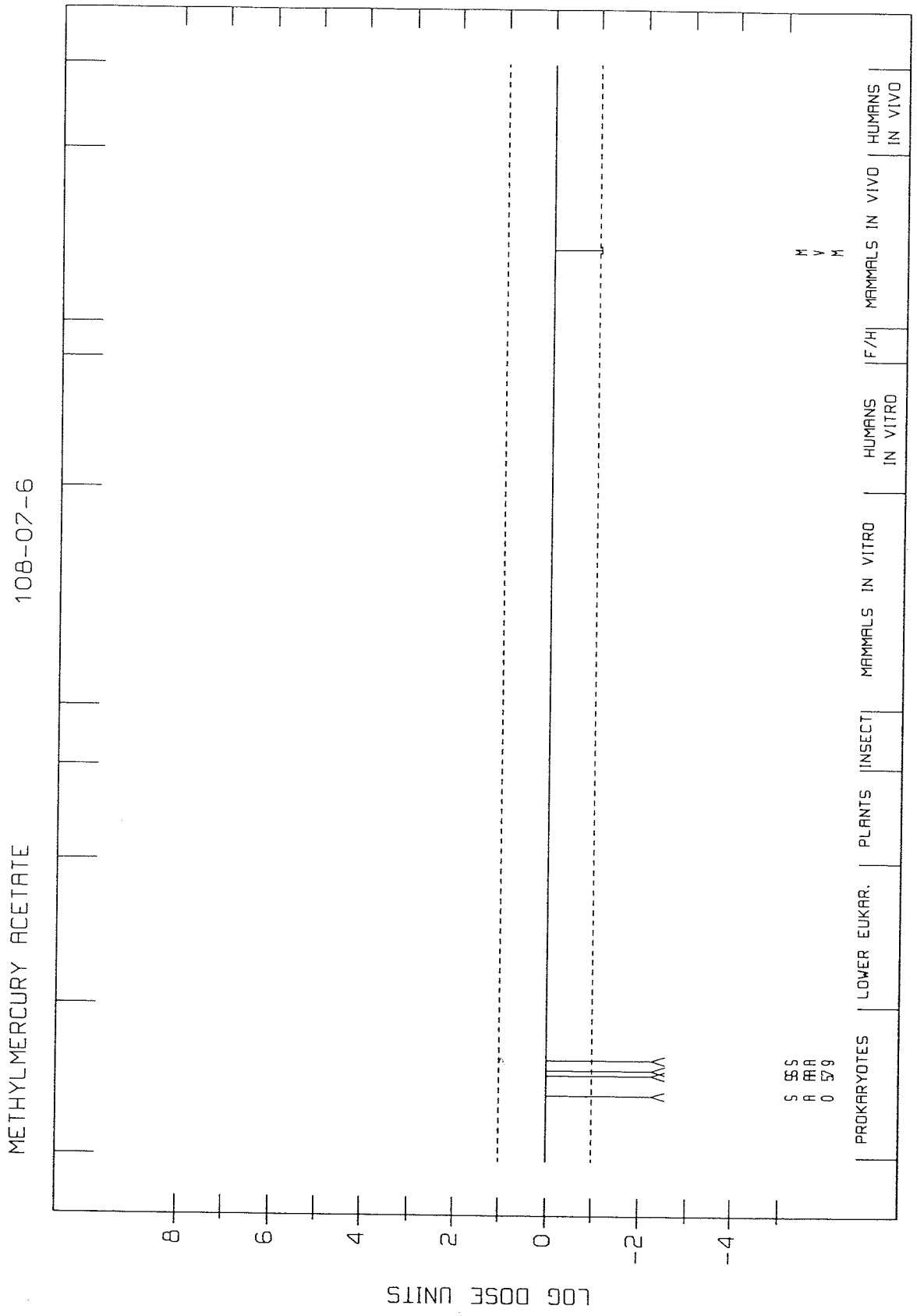
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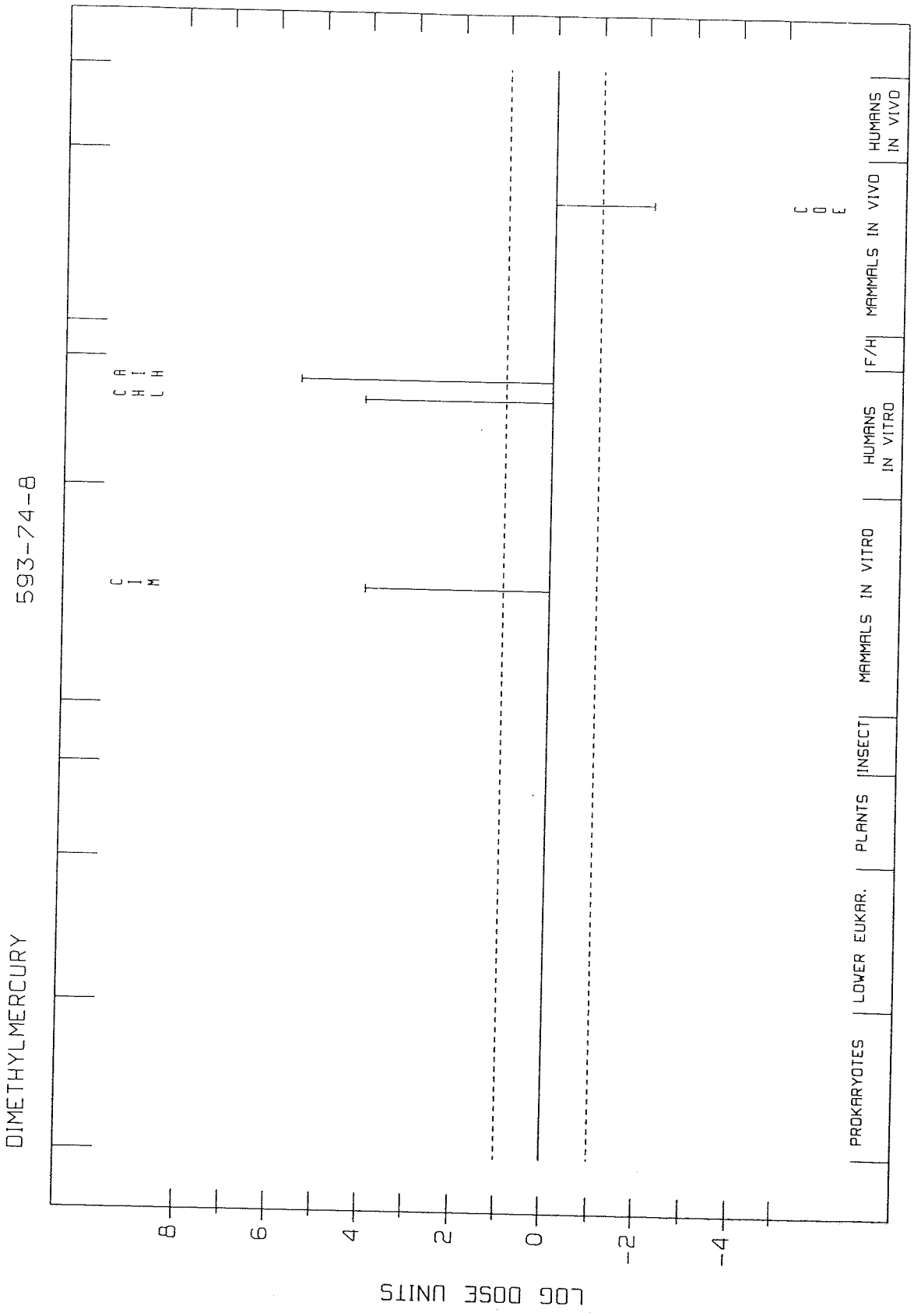
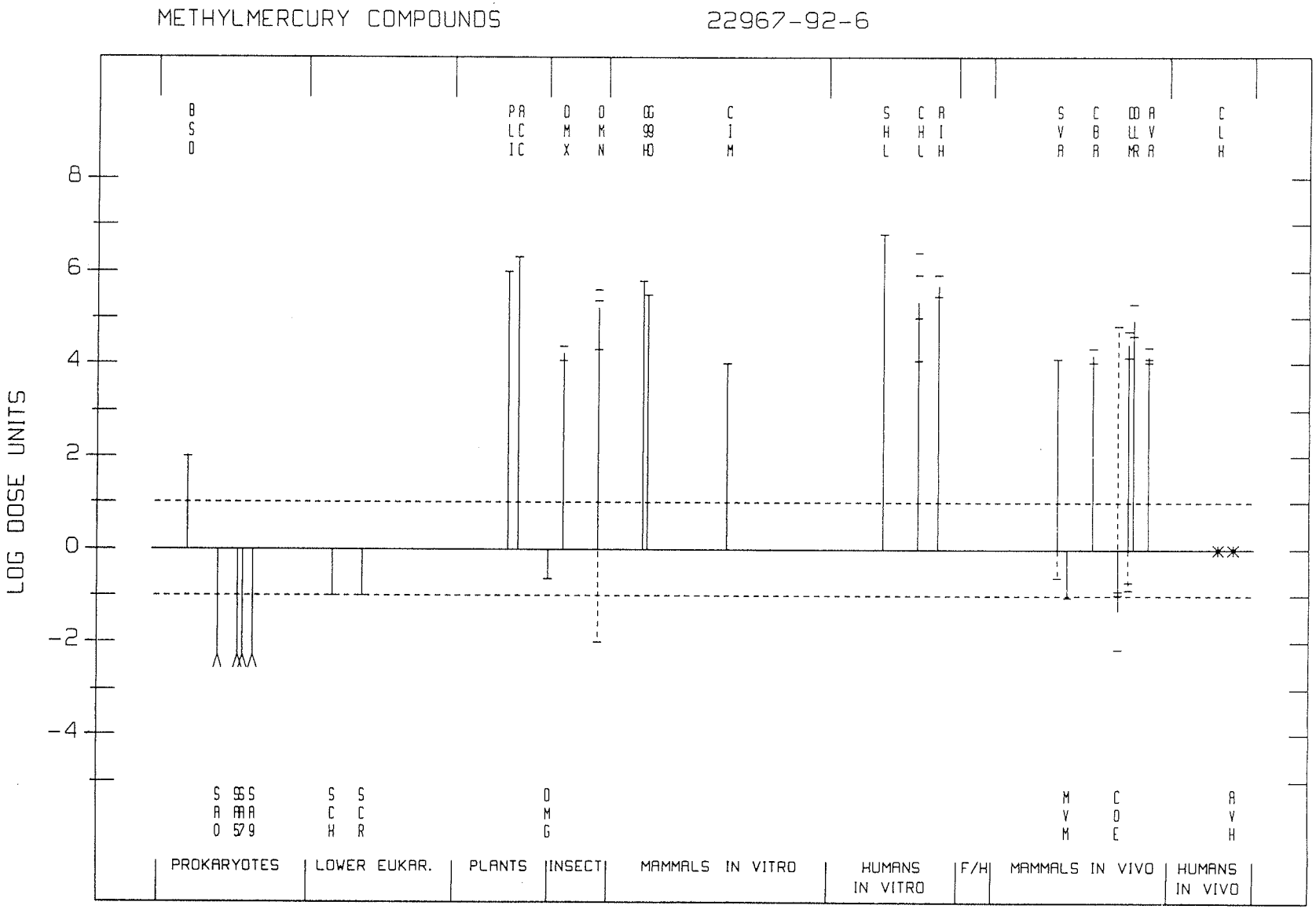
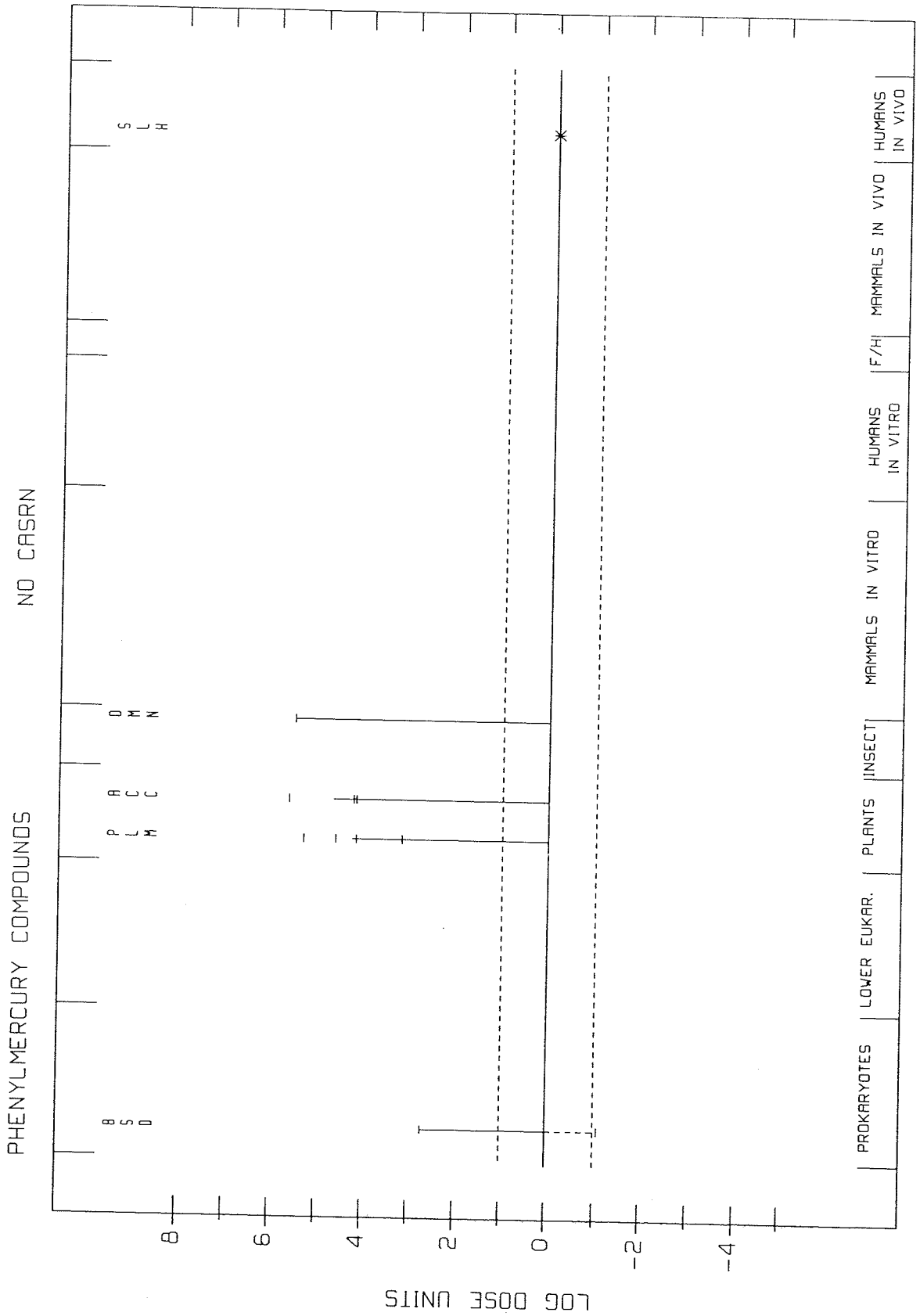
108-07-6
LOG DOSE UNITS
8
6
4
2
0
-2
-4
PROKARYOTES
LOWER EUKAR.
PLANTS
INSECT
MAMMALS IN VITRO
HUMANS IN VITRO
F/H
MAMMALS IN VIVO
HUMANS IN VIVO


DIMETHYLMERCURY
593-74-8
C A
H I
L H
C
O
E
PROKARYOTES
LOWER EUKAR.
PLANTS
INSECT
MAMMALS IN VITRO
HUMANS IN VITRO
F/H
MAMMALS IN VIVO
HUMANS IN VIVO
8
6
4
2
0
-2
-4
LOG DOSE UNITS


METHYLMERCURY COMPOUNDS
22967-92-6
LOG DOSE UNITS
8
6
4
2
0
-2
-4
BSD
PRLIC
DMX
DMN
CIM
SHL
CHL
RIH
SVR
CBR
AVR
CLH
SAO
SCH
SCR
DMG
MVM
COE
AVH
PROKARYOTES
LOWER EUKAR.
PLANTS
INSECT
MAMMALS IN VITRO
HUMANS IN VITRO
F/H
MAMMALS IN VIVO
HUMANS IN VIVO


PHENYLMERCURY COMPOUNDS
NO CASRN
B S D
P L C M C
D M N
S L H
PROKARYOTES
LOWER EUKAR.
PLANTS
INSECT
MAMMALS IN VITRO
HUMANS IN VITRO
F/H
MAMMALS IN VIVO
HUMANS IN VIVO
8
6
4
2
0
-2
-4
LOG DOSE UNITS