NRA-SOUTHERN 180

TECHNICAL DEPARTMENT
SCIENCE GROUP

NRA

National Rivers Authority

Southern Region

NATIONAL RIVERS AUTHORITY
SOUTHERN REGION

TECHNICAL SERVICES
SCIENCE GROUP

AN ASSESSMENT OF THE NRA ALGAL GROWTH INHIBITION TEST.

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PART 1. THE NRA.

1.1 INTRODUCTION.

The Water Act 1989 brought about the restructuring of the water industry and the creation of the National Rivers Authority. The NRA is a public body with statutory responsibilities to protect and improve the water environment in England and Wales, and provide protection against flooding from rivers and the sea.

1.1 STRUCTURE.

The Authority has a Head Office in Bristol and London with operational activities being carried out in 8 regions, originally 10, created from the regulatory functions of the former Regional Water Authorities. The NRA's strategy and policies are formulated by a board of fifteen members appointed by Ministers of the Department of the Environment, the Welsh Office and the Ministry of Agriculture, Fisheries and Food. Development of these Policies is carried out by the Head Office Executive Team and implementation within regions is co-ordinated by the Operations Team. In addition the NRA has 3 statutory committees in each region; namely the Regional Flood Defence Committee, the Regional Rivers Advisory Committee and the Regional Fisheries Advisory Committee.

1.2 FUNDING.

The NRA is funded jointly by levied charges and Government grants. Charges include water abstraction fees, flood defence levies, and both fishing licence and navigation fees. The NRA has also recently introduced a charging scheme for discharges to the aquatic environment.

1.3 CORE FUNCTIONS.

The Authority's main aims and responsibilities are, -

i) Water Resource Management.

The NRA seeks to assess, manage conserve and improve water resources including aquifers through sustainable development plans. This area has included the work to overcome problems of low flows in some catchment areas.

ii) Environmental Quality and Pollution Control.

Since the introduction of the 1974 Pollution Control Act, the release of potentially polluting effluents into UK receiving waters has been controlled by means of discharge consents. The consents are legal agreements between the discharger and the Authority which in many cases stipulate physical and chemical constraints in the effluent. In addition to developing this the NRA has improved procedures for responding to pollution emergencies and undertaken national Water Quality Surveys, which has helped in monitoring standards and setting objectives. In addition the NRA has put into practice a Polluter Pays policy which has increased the financial responsibility of polluters.

iii) Flood Defence.

The Authority is responsible for providing adequate protection from the flooding of rivers and the sea. This includes the management of rivers and their flood plains and the management of sea defences in response to rising sea levels.

iv) Fisheries.

The NRA's work in the area of maintenance, development and restoration of fisheries includes legal duties, stock assessment, poaching prevention, and response to fish kills and disease outbreaks. These responsibilities concern inland fisheries and include coastal waters extending six nautical miles.

v) Recreation, Conservation & Navigation.

The Authority seeks to promote conservation and recreation both internally and externally through it's work, and has important powers and duties in respect of this and navigation.

The NRA's core functions are backed-up by a range of support services, which include legal services, research and development programs, and information systems.

PART 2. BIOLOGY COMPONENT.

From the 2nd of August 1993 my industrial placement year was spent with the Biology and Ecotoxicology Sections of the Southern Region Science Group. At the Waterlooville laboratory the Science Group consists of the Investigations, Quality Audit, Ecotoxicology Sections and Biology which has an additional team at Canterbury. Each section offers a variety of services to the Public and Private sectors and other Departments, generally within the region.

The Biology Section offers the following services; -

- Biological assessment and classification of water quality;
- Biological impact assessment of point, intermittent and diffuse inputs;
- Tracing of pollution sources;
- Sampling and analysis of freshwater and marine macroinvertebrates;
- Aquatic plant surveys;
- Assessment of conservation value;
- Identification of algal blooms including problem species;
- Fish pathology;
- Microbiological analyses including faecal coliforms, streptococci (in waters, sediments and tissues), *Salmonella sp.*, coliphages and tracer organisms.

During the placement period I was involved in the following areas of work ; -

i) Collection and analysis of benthic macroinvertebrate samples for routine monitoring purposes and special investigations. The NRA uses this method for the assessment of river water quality. Changes in water quality affect the diversity of species and the number of individuals present at a site. Mild pollution exerts a differential effect on the members of a macroinvertebrate community. As pollution levels increase, species numbers decline and the resulting lack of competition can lead to a proliferation of those pollution tolerant species. The nationally recognised system for the assessment of biological quality of rivers is the BMWP score (Chesters, 1980). All taxonomic families present are listed and given a score value. These scores are added to give a total cumulative score which is used in comparative studies. In addition to this the NRA uses the ASPT (Average Score Per Taxon), which is a measure of the average sensitivity of the taxa present. The ASPT is generally seen as a more reliable measure of water quality since it is not as sensitive to sampling variation as the BMWP system.

ii) bacteriological analysis of water samples for compliance with EC Bathing Waters Directive. The primary indicators of microbiological contamination are the coliform group, which are found in large numbers in the faeces of man and other warm blooded animals. Quantitative analyses of these organisms in samples is used to determine the presence and extent of contamination.

iii) fish examinations in relation to mortalities and suitability for transfer between water bodies. Diseases of fish, including some parasitic infections are catergorised as either A, B, C, or D under the Diseases of Fish Acts 1937 and 1983. Fish which upon examination are found to have at least one category A disease are not transferrable between water bodies, which reduces the risk of the disease spreading. The examination procedure includes an external check, noting any lesions or abnormalities, eg ulcers, scale loss, deformities or damaged fins. The following organs are then examined: gills, eyes, visceral organs, alimentary canal, swim-bladder, heart and reproductive organs.

iv) collection of marine organisms (mussels and oysters), in relation to EC Dangerous Substances Directive. Tissues of the marine organisms are macerated for subsequent analysis. Biological analysis involves the enumeration of coliform bacteria. Samples of the tissue are also prepared for further chemical analysis.

PART 3. ECOTOXICOLOGY COMPONENT.

3.1 Introduction.

To date the NRA has controlled the release of potentially polluting effluents by chemical discharge consents. These consents specify maximum permitted levels of individual chemicals in the effluent. Monitoring compliance with these consents via chemical analysis can be complicated and expensive particularly when dealing with complex effluents, in which individual constituents are often unknown, and can be numerous. Lack of information on toxicity of both the combined and individual chemicals further increases the problem. It is for this reason that the NRA has developed a protocol for setting a toxicity based consent which is being pilot tested for the next two years and could become part of the UK water quality management procedure by 1996. This would allow the toxicity of complex effluents to be controlled.

There are a number of methods currently being developed, which will form a battery of tests to be used in assessing an effluents' toxicity. The project I undertook during my placement year involved the setting up of the NRA algal growth inhibition test. The Water Research Centre, under contract to the NRA, has produced a protocol for the test which has been submitted in various draft forms during the project. The project's aim has been to assess the protocol and gather data on reference toxicants. Reference toxicants are used in assessing variations in sensitivity of test organisms over time thereby acting as a quality control. In addition, some of the tests performed included samples either as 100% bioassays or as a dilution series.

3.2 Principles of the test.

In the tests performed exponentially growing unialgal cultures were exposed to a range of concentrations of the reference toxicant (and effluent sample when included) over several generations under defined conditions. The inhibition of growth (or growth rate), in relation to a control culture, is determined in a static system over 72 hrs. The different effluent concentrations in an appropriate range will, under otherwise identical test conditions, exert different toxic effects on algal growth (if the effluent is toxic). These will range from no inhibition of growth at lower test concentrations to complete inhibition at higher test concentrations. The data are used to determine the concentration which inhibits algal growth by 50 % after 72 hrs in relation to the control. This is referred to as the 72 hr-IC50.

3.3 Methods.

3.3.1 Apparatus.

The following apparatus is used:

- an orbital incubator which is capable of maintaining cultures at a constant 20-25^{o}C at +/- 2^{o}C with continuous illumination in the spectral range 400 to 700 nm;
- a Coulter counter for measuring cell density in samples of the test cultures;
- 250 ml conical test flasks with air permeable stoppers (non-absorbant cotton wool);
- apparatus for membrane filtration;
- an autoclave;
- a pH meter and probe.

NB/ detailed specifications of the apparatus appears on page 69 of the protocol. The specifications were followed exactly with the sole exception that filtration, when necessary was performed using cellulose filters with mean pore diameter 0.45 μm.

3.3.2 Test procedures.

The methods for the tests were obtained from the WRC protocol and adhered to specifically with the exception of slight deviation from the method described on page 73 of the protocol. The protocol, on page 73, describes the preparation of nutrient dilutions in the test series: it states that freshly prepared media should be added to volumetric flasks which already contain the range of volumes of test substance necessary for the dilution series and goes on to say that reduced algal growth rates may occur due to this method of preparation.

In the tests reported the nutrient stock solutions were added to the series prior to the addition of any other constituents of the test mixtures. Therefore each test mixture contains the same concentrations of nutrients, which discounts the possibility of reduced growth rates due to limiting nutrient concentrations.

The tests were performed on exponentially growing cultures of the freshwater algae *Scenedesmus subspicatus* which were obtained from the Culture Centre of Algae and Protozoa (CCAP 276/20). Cultures were regularly sub-cultured to fresh media to sustain exponential growth. This was done using a 1:100 culture to fresh media dilution.

The pre-cultures were maintained in constant conditions in a Gallenkamp growth cabinet (lighting-1700 lux; temp-20°C +/-1°C). Three-day old cultures were used to provide the inoculant at the beginning of each test. For the three day growth period the pre-culture remained in the orbital incubator under conditions identical to those used for the following test. Cell density in the pre-cultures ranged from 293000 cells ml^{-1} to 724000 cells ml^{-1}. The volume of inoculum added to each test substance stock solution was calculated such that an initial cell density of 10000 cells ml^{-1} was achieved in the test mixtures.

During the course of the test, aliquots of the test mixtures were removed at 24-hourly intervals to measure cell density. A coulter counter was used to perform counts on the samples removed. The procedure for this was as follows:

i) 35 ml aliquots of *ISOTON* (see appendix F) were dispensed into clean, acid rinsed 50 ml beakers with a metered peristaltic pump.

ii) a 5 ml sample of each test mixture was removed with a sterile pipette and placed in one of the beakers containing 35 ml of *ISOTON*.

iii) the sterile pipette is replaced after each sample is removed to prevent contamination of the test mixtures.

iv) after a number of consecutive samples had been taken it was possible that settling of the algae had occurred so the orbital shaker was switched on intermittently to mix the cultures.

v) the diluted sample, (1:8), was placed on the Coulter sampling stand and the stand raised. The mixture was well stirred before performing 3 consecutive counts to obtain a mean.

The set-up procedure for the Coulter counter is given in appendix F.

3.4 Results of reference toxicants.

3.4.1 Phenol results.

In table 1 below, percentage cumulative inhibition, respective to the control appears against phenol concentration. As the protocol suggested, a geometric concentration range was used in preliminary tests to investigate an ideal concentration range for the reference toxicant. The preliminary tests were prone to experimental error. The results for the tests dated 02.03.94 and 08.03.94 display this. For this reason these results were left out when constructing the control chart for phenol toxicity to *S.subspicatus* which appears on page 14.
Individual test results are displayed graphically in appendix A, the following appear :

i) dose response curves ;

ii) percentage cumulative inhibition against phenol concentration graphs.

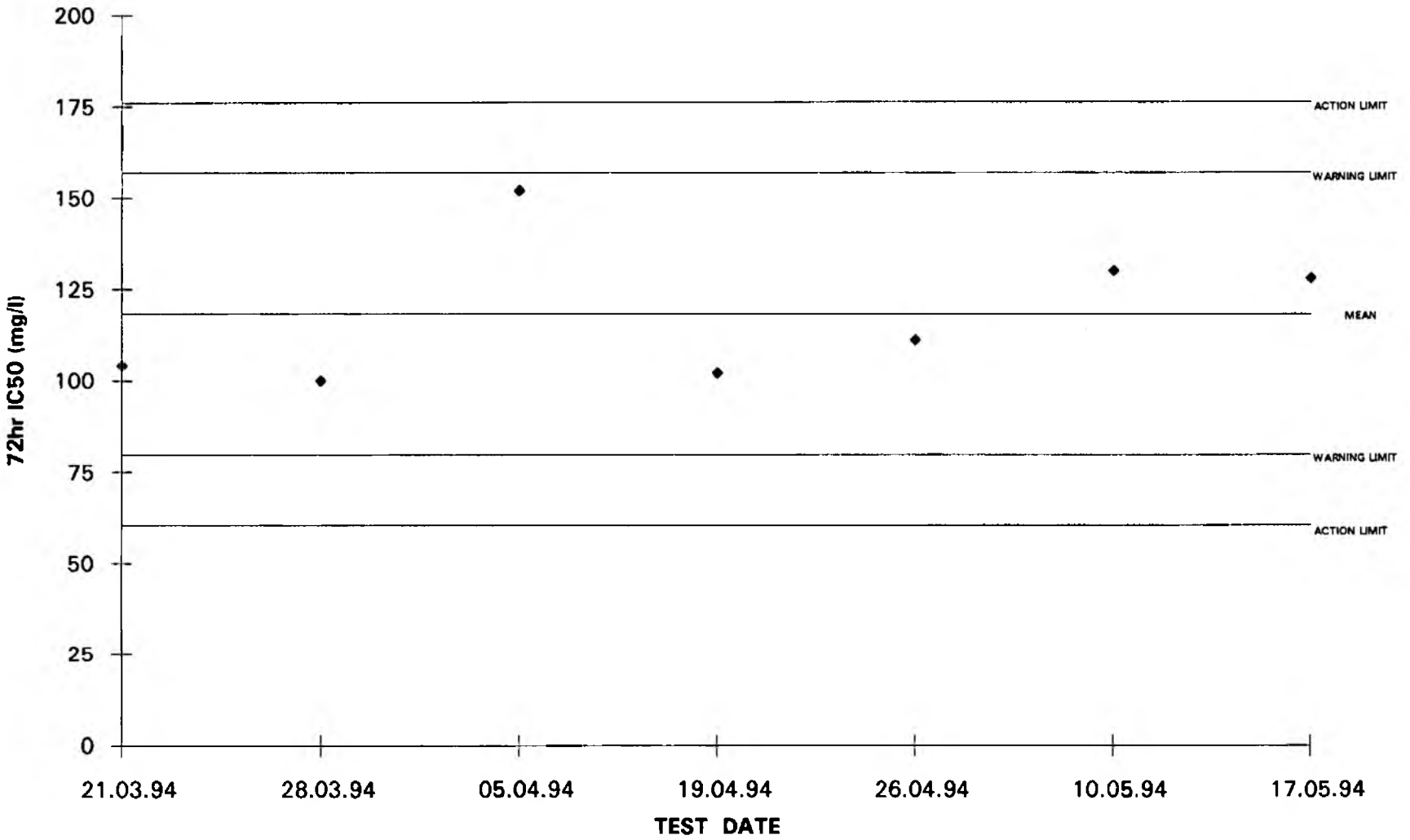
Table 1. Growth inhibition (%) of *S.subspicatus* by phenol.

Concn. mg/l	02.03.94	08.03.94	21.03.94	28.03.94	05.04.94	19.04.94	26.04.94	10.05.94	17.05.94
0.1	0.0	–	–	–	–	–	–	–	–
0.32	0.0	–	–	–	–	–	–	–	–
1.0	38.8	–	–	–	–	–	–	–	–
3.2	0.0	–	–	–	–	–	–	–	–
10.0	15.3	–	–	–	–	–	–	–	–
32.0	0.0	–	–	–	–	–	–	–	–
50.0	–	–	29.4	37.5	10.6	29.1	24.1	–	24.0
100.0	0.0	61.8	49.3	45.0	31.1	44.4	39.1	38.7	31.2
150.0	–	–	–	56.1	–	60.3	59.1	–	55.0
200.0	–	45.2	74.1	74.1	59.6	80.9	80.7	69.8	69.4
250.0	–	68.2	–	–	–	–	–	–	–
300.0	–	92.9	86.2	90.9	83.4	–	–	91.4	–
320.0	>100.0	–	–	–	–	–	–	–	–
400.0	–	>100.0	–	–	–	–	–	>100.0	–
500.0	–	>100.0	–	–	–	–	–	–	–

3.4.2 Control chart of phenol toxicity to *Scenedesmus subspicatus*.

The 72hr-IC50's are tabulated and the standard deviation of the data set calculated, (see table 6, appendix D). A control chart of the individual 72hr-IC50's was produced (next page) with warning and action limit bars at +/- 2 and 3 standard deviations respectively. The chart does not include any 72hr-IC50 results from the first two phenol tests as it was impossible to derive one from the inaccurate data.

CONTROL CHART FOR PHENOL TOXICITY TO SCENEDESMUS SUBSPICATUS



3.4.3 Zinc results.

Table 2 below shows the percentage cumulative inhibition of *S.subspicatus* against zinc concentration for the tests performed. The results of the first two preliminary tests dated 08.02.94 and 21.02.94 show experimental error, and were ignored when constructing a control chart of zinc toxicity to *S.subspicatus* which appears on page 18. Over the project period the concentration range used has been narrowed down to: 0.5, 0.75, 1.0 and 1.25 mgl^{-1} zinc.

Individual test results appear in appendix B as :

i) dose response curves ;

ii) percentage cumulative inhibition against zinc concentration graphs.

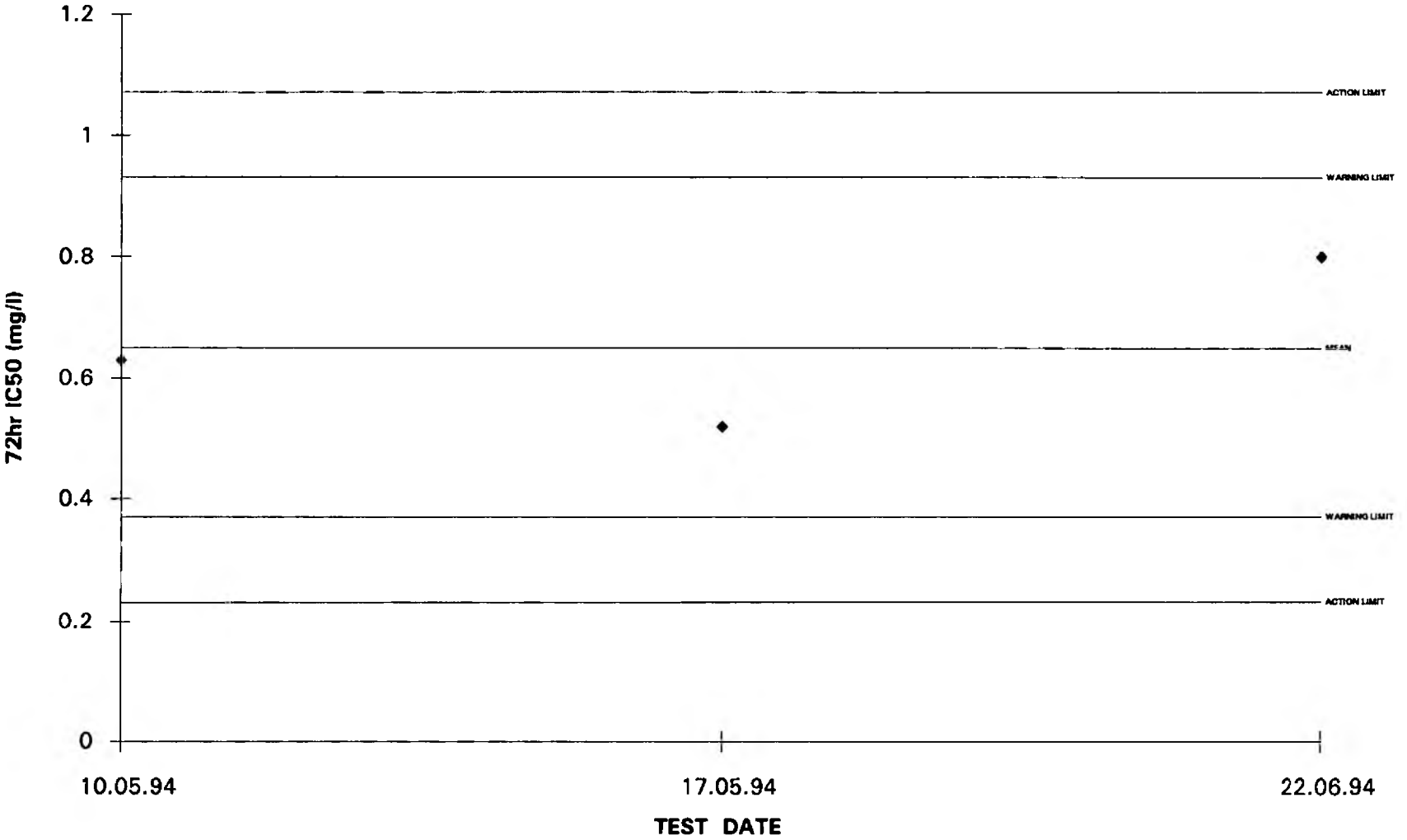
Table 2. Growth inhibition (%) of *S.subspicatus* by zinc.

Concn. mg/l	08.02.94	21.02.94	10.05.94	17.05.94	22.06.94
0.0032	0.0	–	–	–	–
0.01	15.7	–	–	–	–
0.032	45.7	–	–	0.0	–
0.1	10.2	–	–	10.8	–
0.32	0.0	–	29.9	17.7	–
0.5	–	–	–	–	29.7
0.75	–	–	–	–	45.2
1.0	0.0	–	63.2	83.1	57.8
1.25	–	–	–	–	79.3
3.2	75.6	–	91.4	93.8	–
10.0	92.9	61.3	>100.0	–	–
32.0	–	63.8	>100.0	–	–
50.0	–	–	–	–	–
100.0	–	58.1	–	–	–
320.0	>100.0	58.9	–	–	–

3.4.4 Control chart of zinc toxicity to *Scenedesmus subspicatus*.

The graph on the next page is a preliminary control chart for zinc toxicity to *S.subspicatus*. The results from tests dated 10.05.94; 17.05.94; and 22.06.94 were used to construct the chart. The standard deviation of the mean 72hr-IC50 was calculated (see table 7, appendix D) and this figure is used to calculate the position of the warning and action limit bars at +/- 2SD and +/- 3SD respectively. As additional tests are performed using zinc the 72hr-IC50 obtained are added to the control chart, thus the mean 72hr-IC50 line and the position of the warning and action limit bars are recalculated accordingly. Thus the accuracy of the predicted 72hr-IC50 improves with the quantity of tests performed.

CONTROL CHART FOR ZINC TOXICITY TO SCENEDESMUS SUBSPICATUS



3.4.5 Results of samples tested.

Receiving waters.

During the test period two receiving waters were tested:

Test date: 19.04.94, see table 3 on page 21.

On this test date samples from six designated sites on the River Yar (eastern) were included as 100% bioassays to investigate the presence of a herbicide in the river which had been reported by Southern Water (see next page for map of the sites). The sites were selected to determine whether or not the herbicide was present at the time of sampling and to determine an entry point if it were present.

Test date: 22.06.94, see table 4 on page 21. Results are also displayed graphically in appendix C.

Serial dilutions of a sample taken from the River Medway, downstream of a biocide spillage. Ideally a more concentrated range of dilutions should have been selected, since only one of the dilution series resulted in an inhibition of growth of more than 50% .

Effluent tested.

Test date: 26.04.94, see table 5 on page 21.

Serial dilutions of the effluent from a sewage treatment works were included. A dilution series was prepared with information of the effluent's toxicity towards Microtox samples supplied by Caroline Rutter. The results suggest experimental error, since the higher concentrations of effluent showed no inhibition to the algal cultures, but the lowest concentration inhibited growth by 9.5 %.

S I T E K E Y			
Number	Location	Watercourse	Grid ref.
1	Burnt House	Eastern Yar	SZ 5832 8529
2	Beacon Alley	Eastern Yar	SZ 5192 8123
3	Newchurch	Eastern Yar	SZ 5597 8584
4	Brading	Eastern Yar	SZ 6068 8630
5	Burnt House	Scotchells Brook	SZ 5824 8513
6	Bathingbourne	Wroxall Stream	SZ 5490 8363

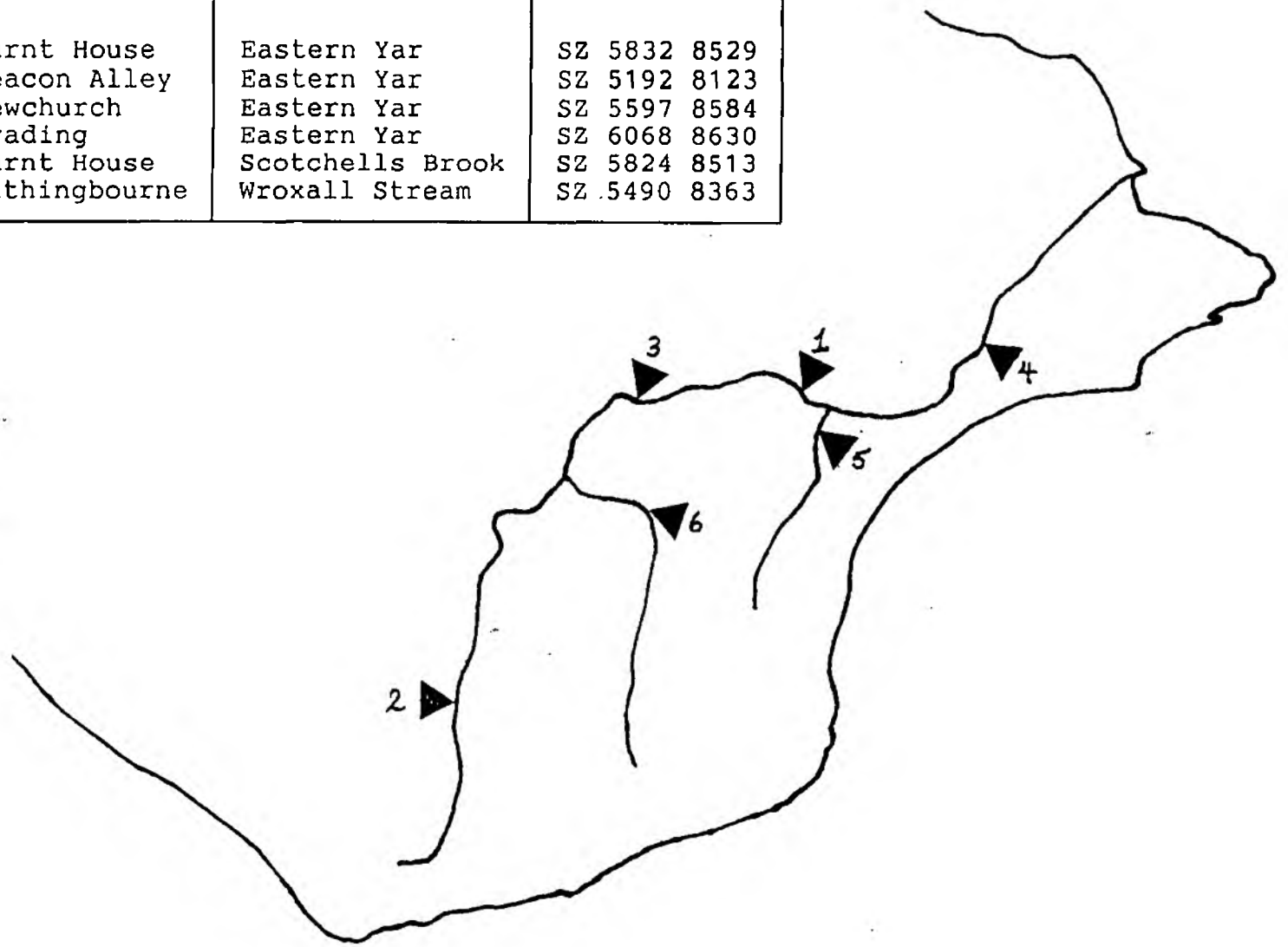


Table 3.
Growth inhibition (%) of *S.subspicatus* by sample bioassays tested on 19.04.94.

Bioassays		19.04.94
	1	3.7
S	2	0.0
I	3	0.0
T	4	0.0
E	5	4.6
	6	0.0

Table 4.
Growth inhibition (%) of *S.subspicatus* by sample from R.Medway (receiving water) tested on 22.06.94.

Percentage receiving water.	22.06.94
0.1	0.0
0.32	0.0
1.0	0.2
3.2	21.5
10.0	35.4
32.0	52.9

Table 5.
Growth inhibition (%) of *S.subspicatus* by the effluent of a sewage treatment works tested on 26.04.94.

% Effluent	26.04.94
0.1	9.5
0.32	4.7
1.0	0.0
3.2	0.0
10.0	0.0
32.0	-

3.5 Discussion.

Test validity.

The protocol for the test states that exponential growth in the control over the test period is required for the 72hr-IC50 derivation to be valid. From the results, it can be seen that this criteria has been met.

Lighting levels.

The 72 hr cell densities in the controls range from 400000 cells ml^{-1} in test 5 to 1800000 cells ml^{-1} in the test dated 17.05.94. This suggests that the difference in light intensity available to the cultures in the respective tests had a direct effect upon the growth rates. The protocol states the required light intensity but goes on to say that if exponential growth is maintained in the control over the test period, sufficient light has been provided.

The lighting in the orbital incubator used for all tests in this project is provided by a bank of 7 fluorescent tubes in the lid of the incubator. In the test dated 21.03.94, only lamps 3 and 5 were switched on, resulting in a final control cell density of 380000 cells ml^{-1}.
In comparison, the test dated 17.05.94 when lamps 1,2,3,5,6 & 7 were switched on, the control cell density reached 1800000 cells ml^{-1} at 72 hrs. The changes in lighting arrangement were made in an attempt to provide uniform lighting across the incubator platform. On all the settings available, the light intensity is greatest in the centre of the platform, and least at the corners. The most uniform lighting setting was found to be with lamps 1,2,6 & 7 switched on throughout the test. To further reduce the affects of varying light intensity replicate flasks were arranged in blocks on the incubator platform (see fig.1, appendix F).

Reference toxicants.

Phenol.

At concentrations above 400 mgl^{-1} phenol there was no growth of the algae. The concentration range used primarily was a geometric concentration series (range finding) which was reduced to 50, 100, 150, and 200 mgl^{-1} towards the end of the project to more accurately assess the 72hr-IC50.

The phenol data displayed in table 1 is consistent from the 21st March and has provided a control chart of the individual 72 hr-IC50's with a small fluctuation about the mean of 120 mgl^{-1}. Only the 72 hr-IC50 from test 7 lies near the +2SD warning line, the rest lie well within the limits. If a reference toxicants 72hr-IC50 falls outside the +/- 3SD action limits the test has failed, that is the tests accuracy is in question. Therefore the control chart acts as a quality control.

Zinc.

The highest concentrations of zinc totally inhibited growth as shown in the dose response curve for the test dated 10.05.94. Above a concentration of 3.2 mgl^{-1} zinc cell density decreased. Therefore, the concentration range of zinc was lowered in test dated 17.05.94. The (zinc) growth curves and cumulative inhibition graph for test dated 17.05.94 show almost 100 % inhibition at 3.2 mgl^{-1} and no inhibition at 0.032 mgl^{-1}. This is an appropriate example of a range finding test as described in the protocol.

Receiving waters.

Statistical analysis was performed on the results from test dated 19.04.94 (table 3). This test included samples from the River Yar (East) as 100% bioassays (the procedure for the bioassay appears on page 77 of the protocol).

A one-way ANOVA compared the means of the samples (see appendix E) and at a significance level of $P=0.05$ (5 %) concluded that there was no difference between the means of the samples. Therefore there is no evidence from the results of this test to suggest the presence of a herbicide at any of the sites sampled.

In the test dated 22.06.94 serial dilutions of a sample obtained from the River Medway following the spillage of a biocide were included (see table 4). A 72hr-IC50 of 23 % sample was obtained from plotting the data on log-normal graph paper (see appendix C for graphically presented results).

Effluent.

The test dated 26.04.94 included the serial dilutions of an effluent sample from a sewage treatment works. The results in table 5 are inconsistent which is likely to be due to experimental error. No graphical representation of the results was included.

3.6 References.

Chesters, R K.(1980). Biological Monitoring Working Party. Technical Memorandum No.19 DOE/WDU.

Johnson, I K. (1983). Laboratory Service For Fish Health Checks. NRA Internal Protocol.

NRA. (1994). Algal Growth Inhibition Tests. R & D draft 420/5/T.

3.7 Appendices.

In sections A, B and C of the appendix the following graphs are displayed ;

i) A graph of cell density (cells ml^{-1}) against time (hrs) for the test mixtures. Each test substance series appears on an individual graph with the control.

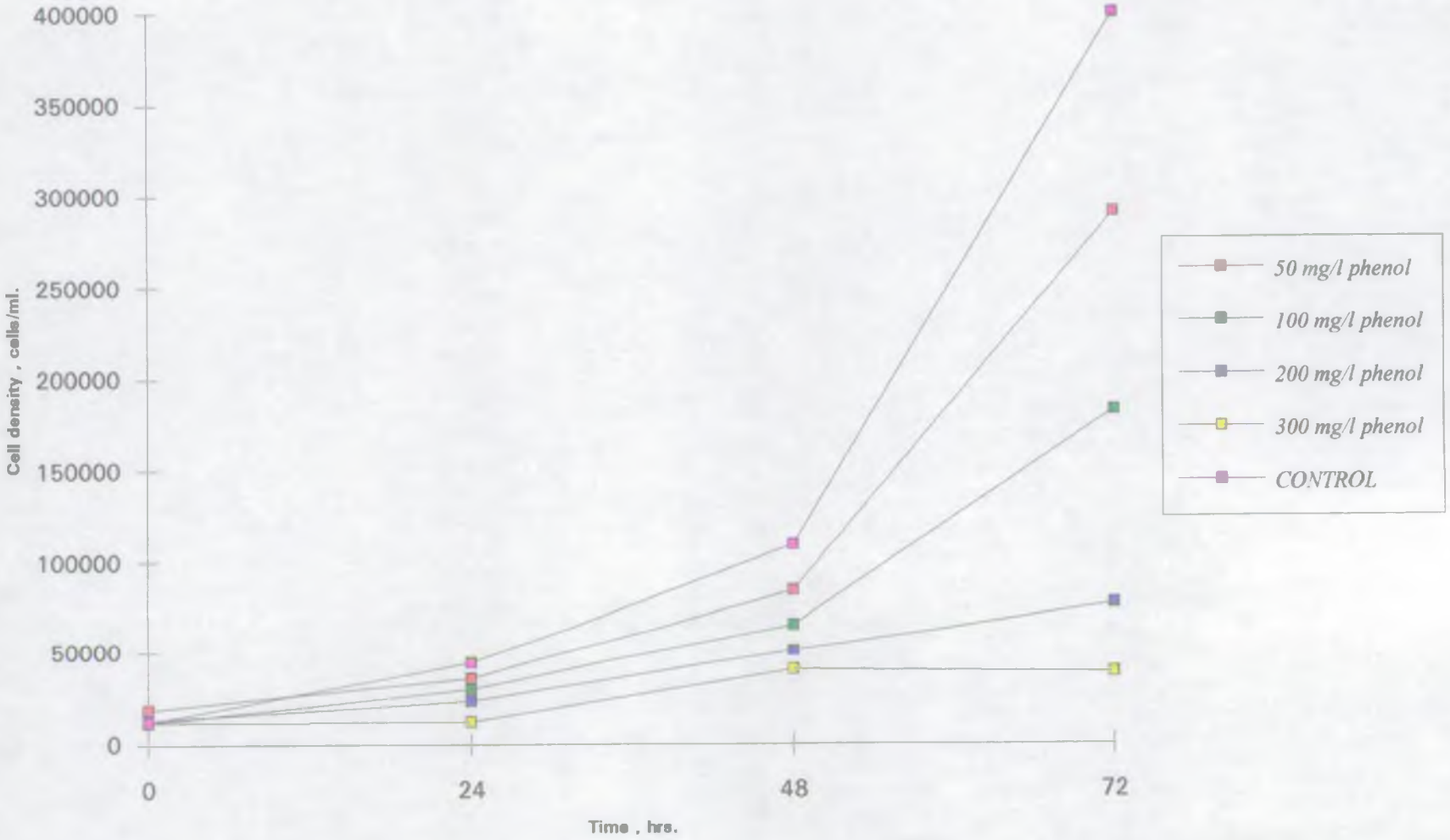
ii) A graph of percentage inhibition against test substance concentration (mgl^{-1}). The 72 hr-IC50 was calculated for each test substance by plotting % inhibition against test concentration on log-normal graph paper and reading off the value for test concentration corresponding to a 50 % reduction in growth rate relative to the control.

Section A: Individual test results for phenol:

NB/ no graphs appear for the first two tests performed due to the inconsistency of the results. This was attributed to experimental error.

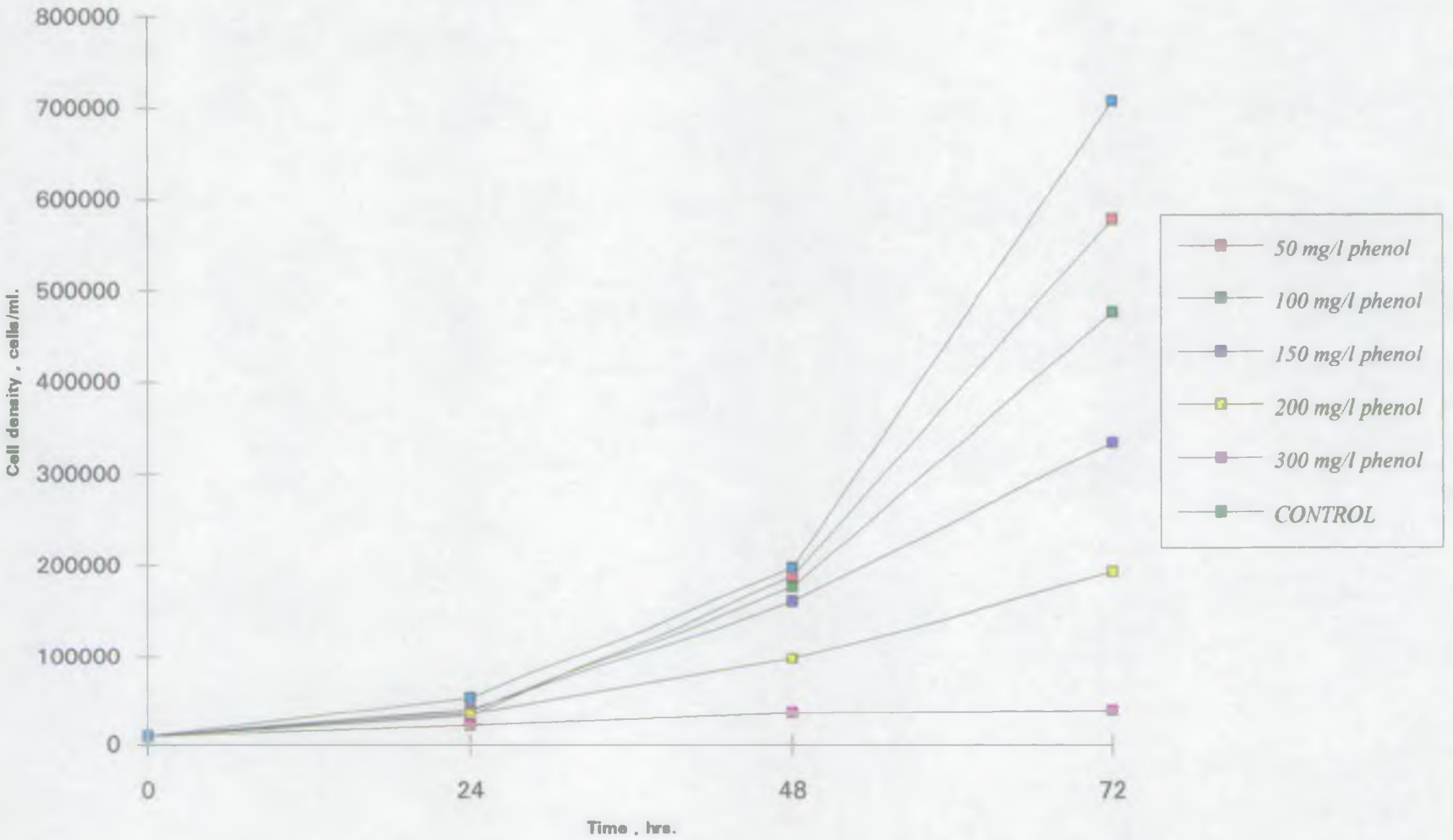
Test date: 21.03.94 Test substance: Phenol

Dose response curve for Scenedesmus subspicatus.



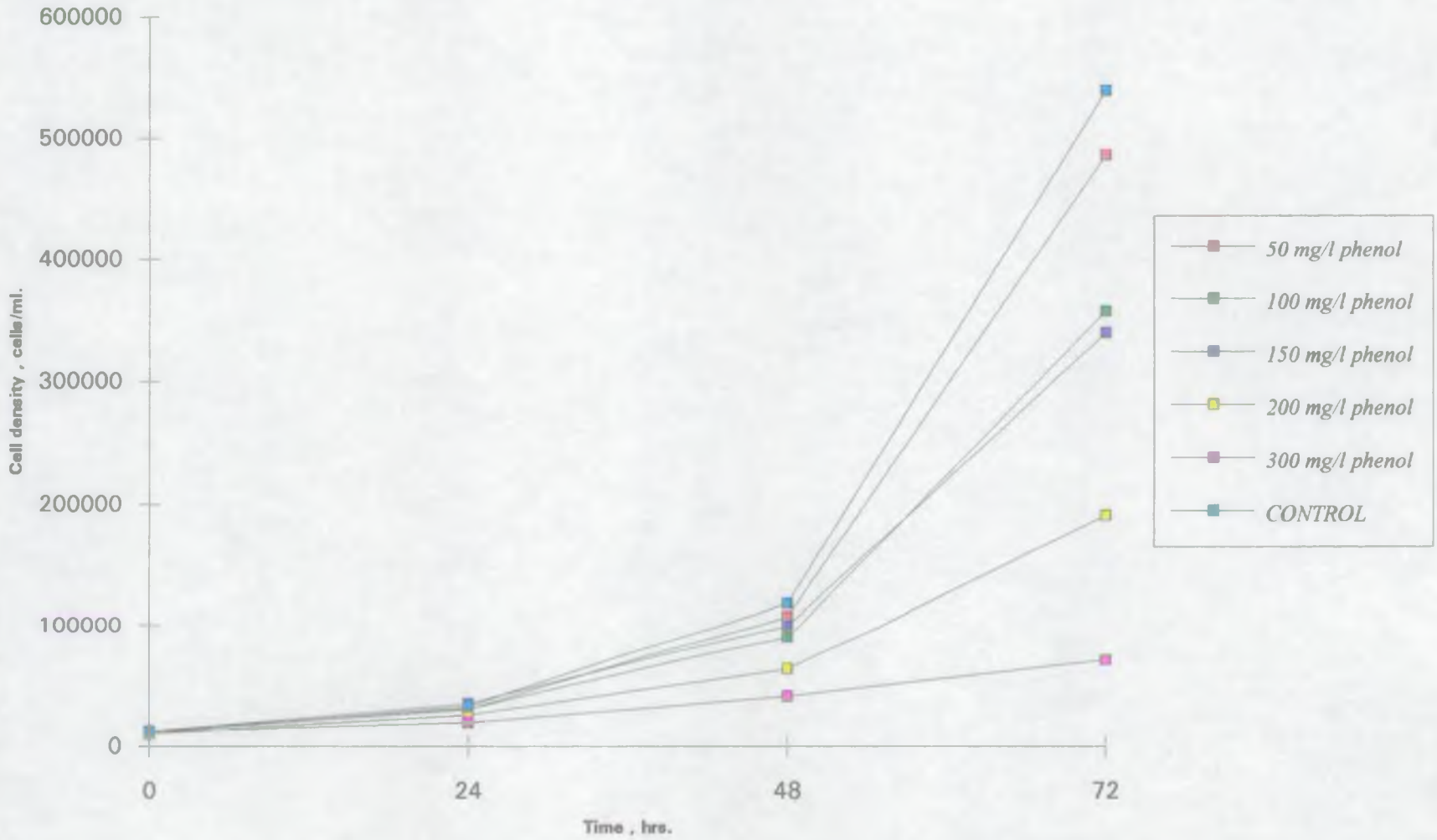
Test date: 28.03.94 Test substance: Phenol

Dose response curve for Scenedesmus subspicatus.



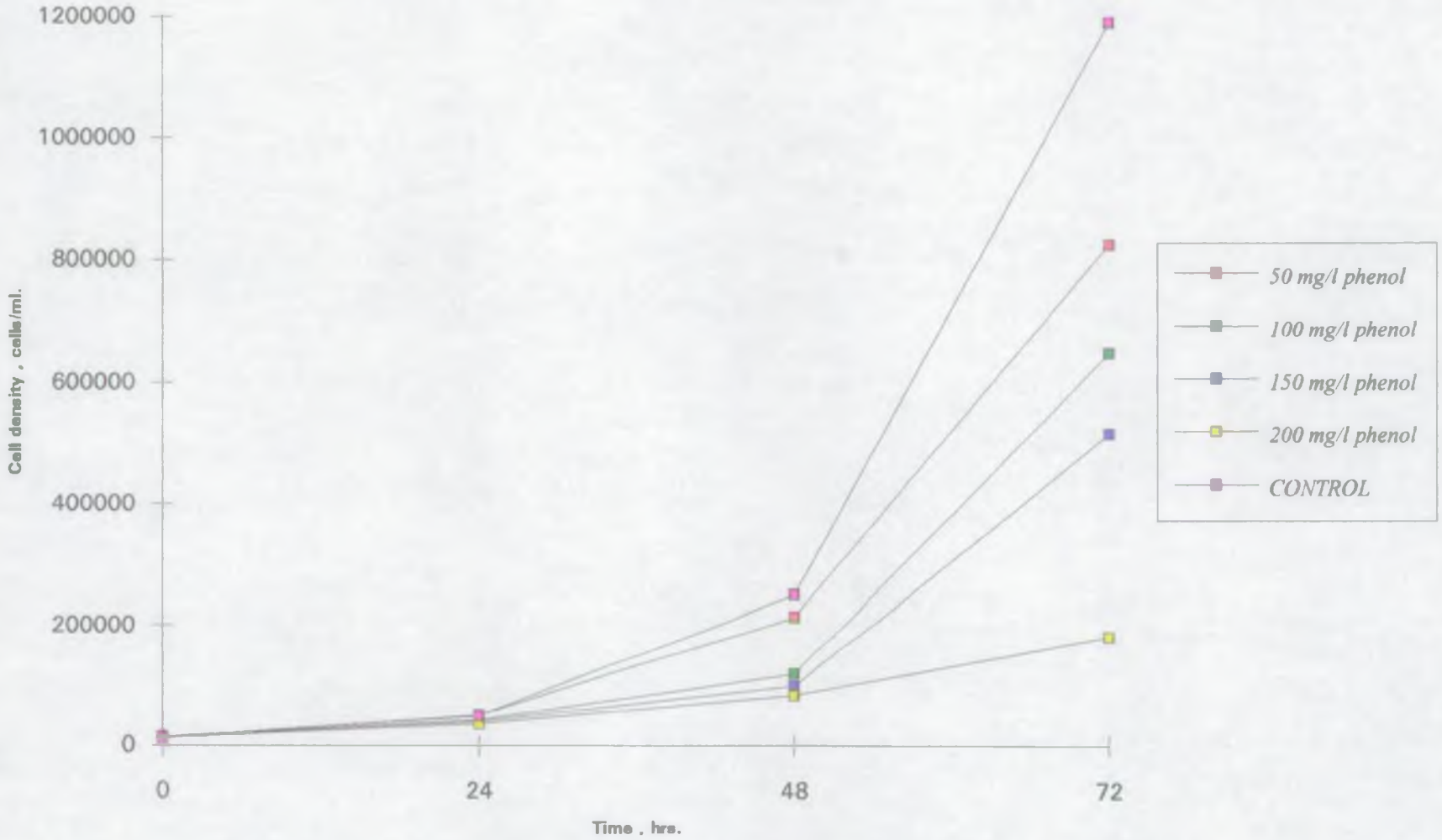
Test date: 05.04.94 Test substance: Phenol

Dose response curve for Scenedesmus subspicatus.



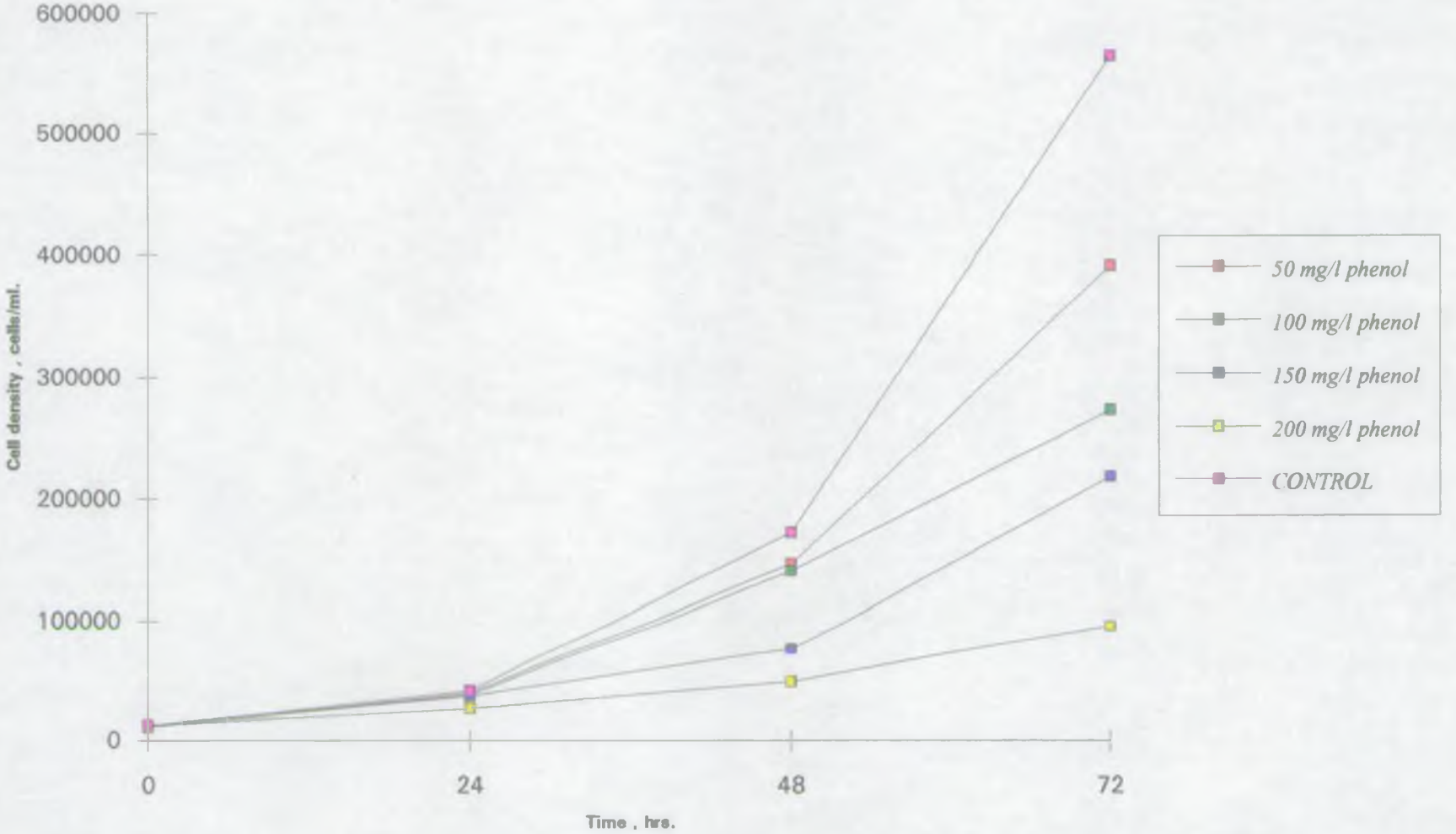
Test date:19.04.94 Test substance: Phenol

Dose response curve for Scenedesmus subspicatus.



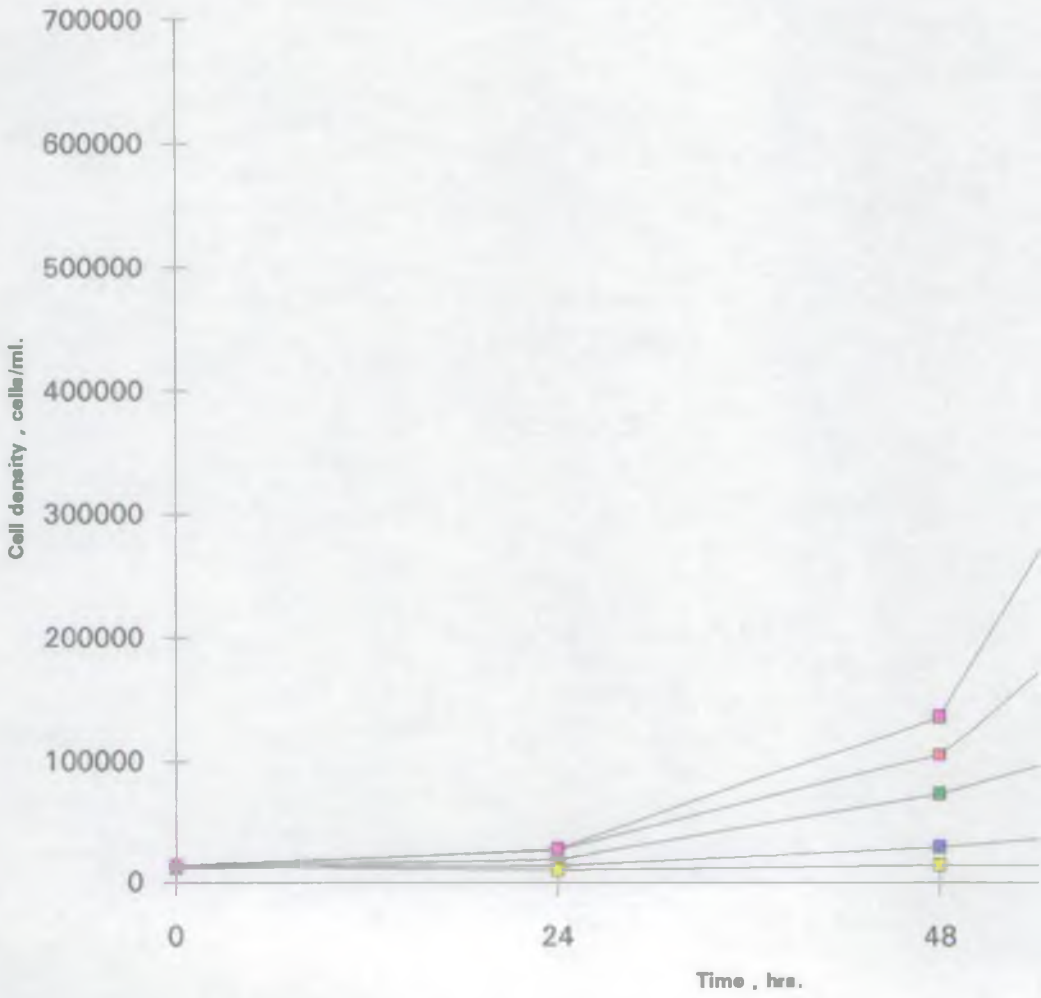
Test date: 26.04.94 Test substance: Phenol

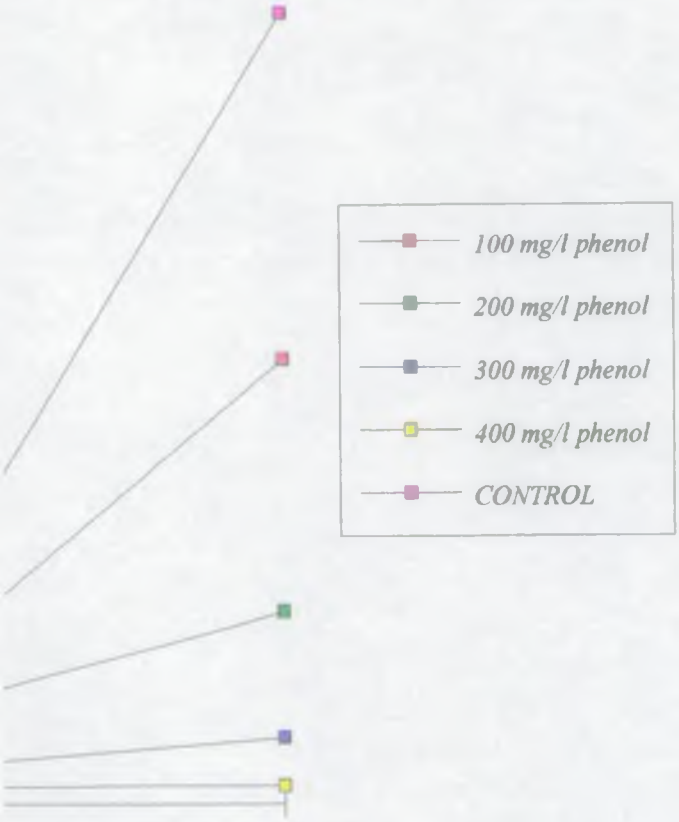
Dose response curve for Scenedesmus subspicatus.



Test date: 10.05.94 Test substance: Phenol

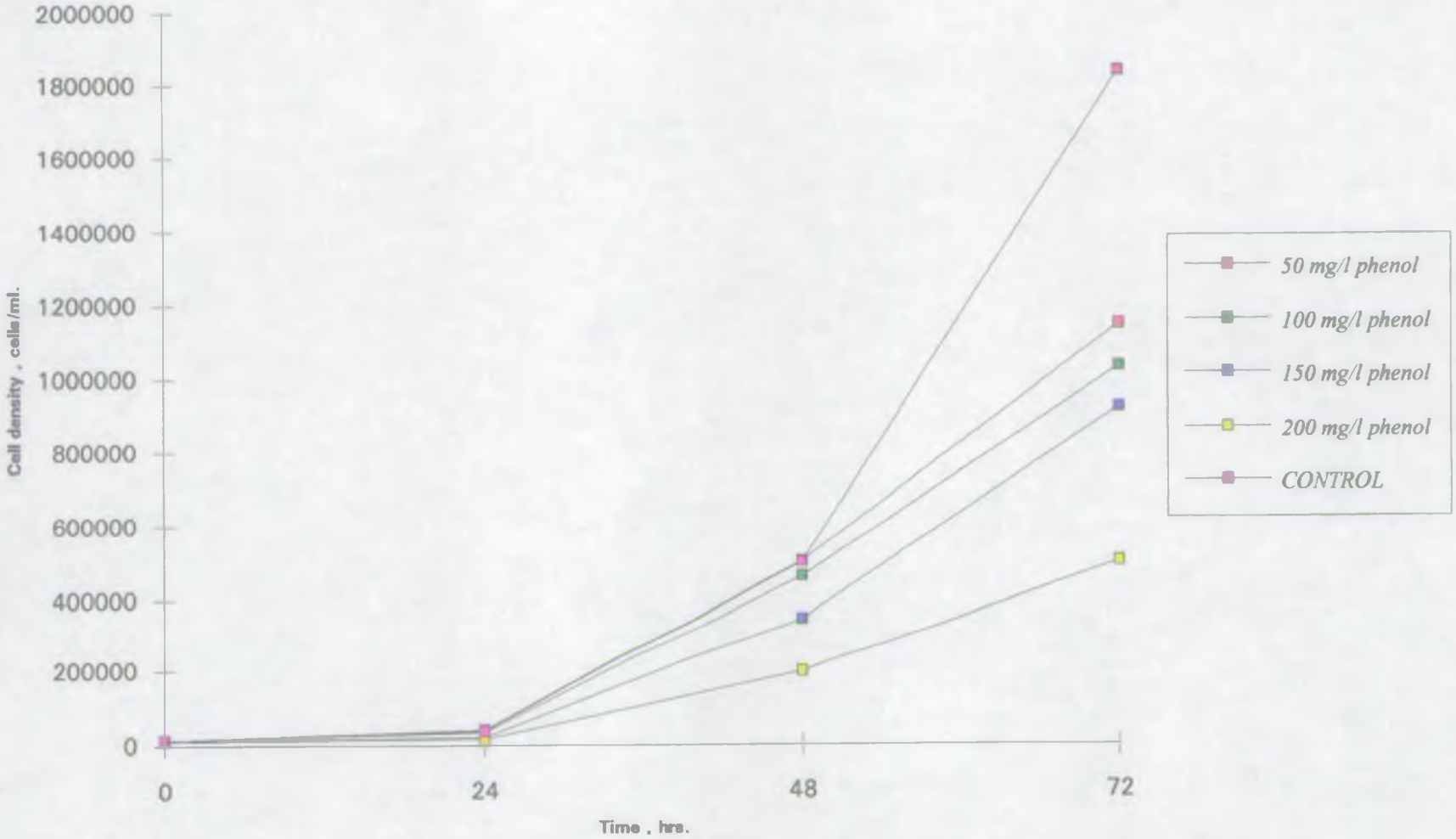
Dose response curve for Scenedesmus subspicatus.

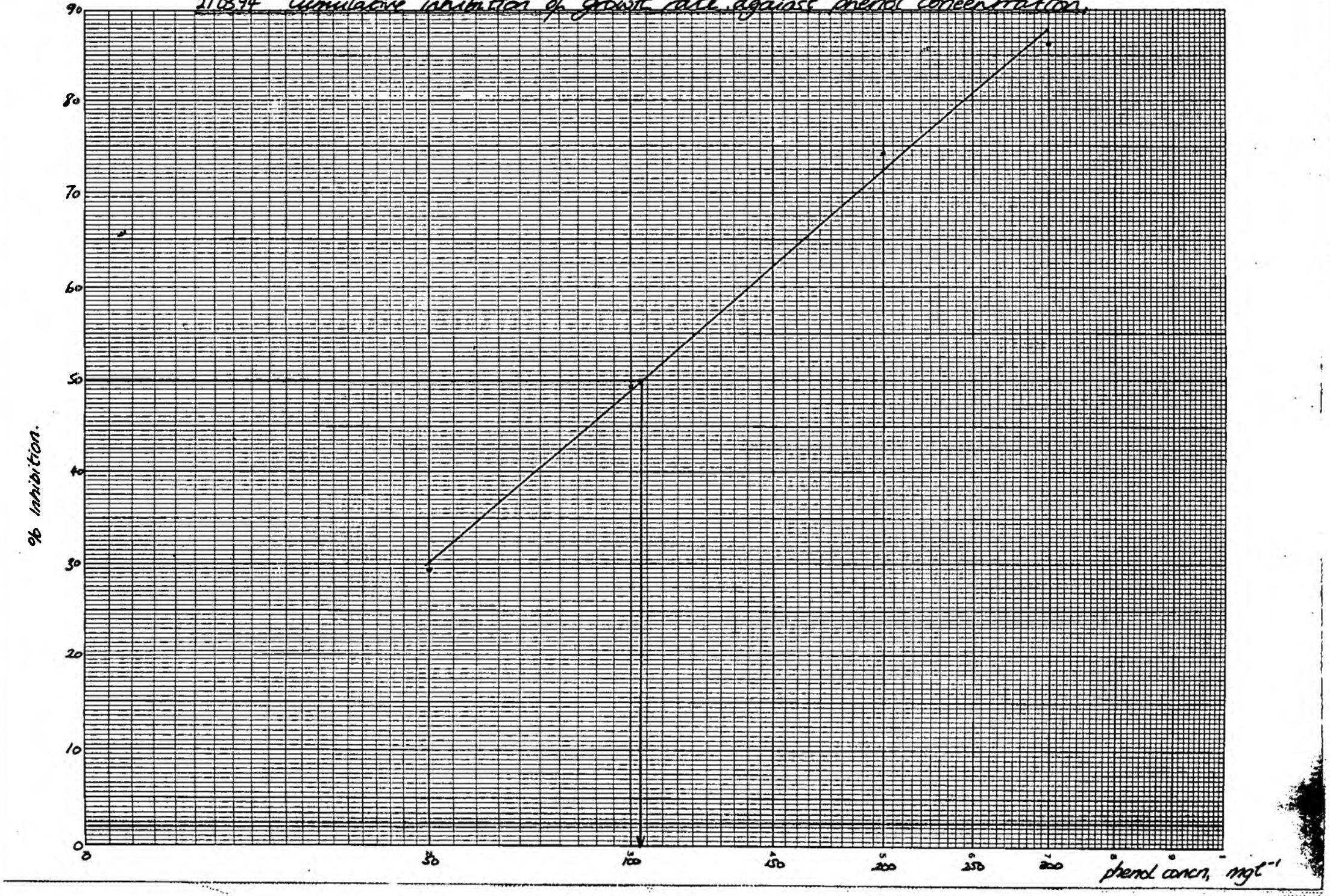



100 mg/l phenol
200 mg/l phenol
300 mg/l phenol
400 mg/l phenol
CONTROL

Test date: 17.05.94 Test substance: Phenol

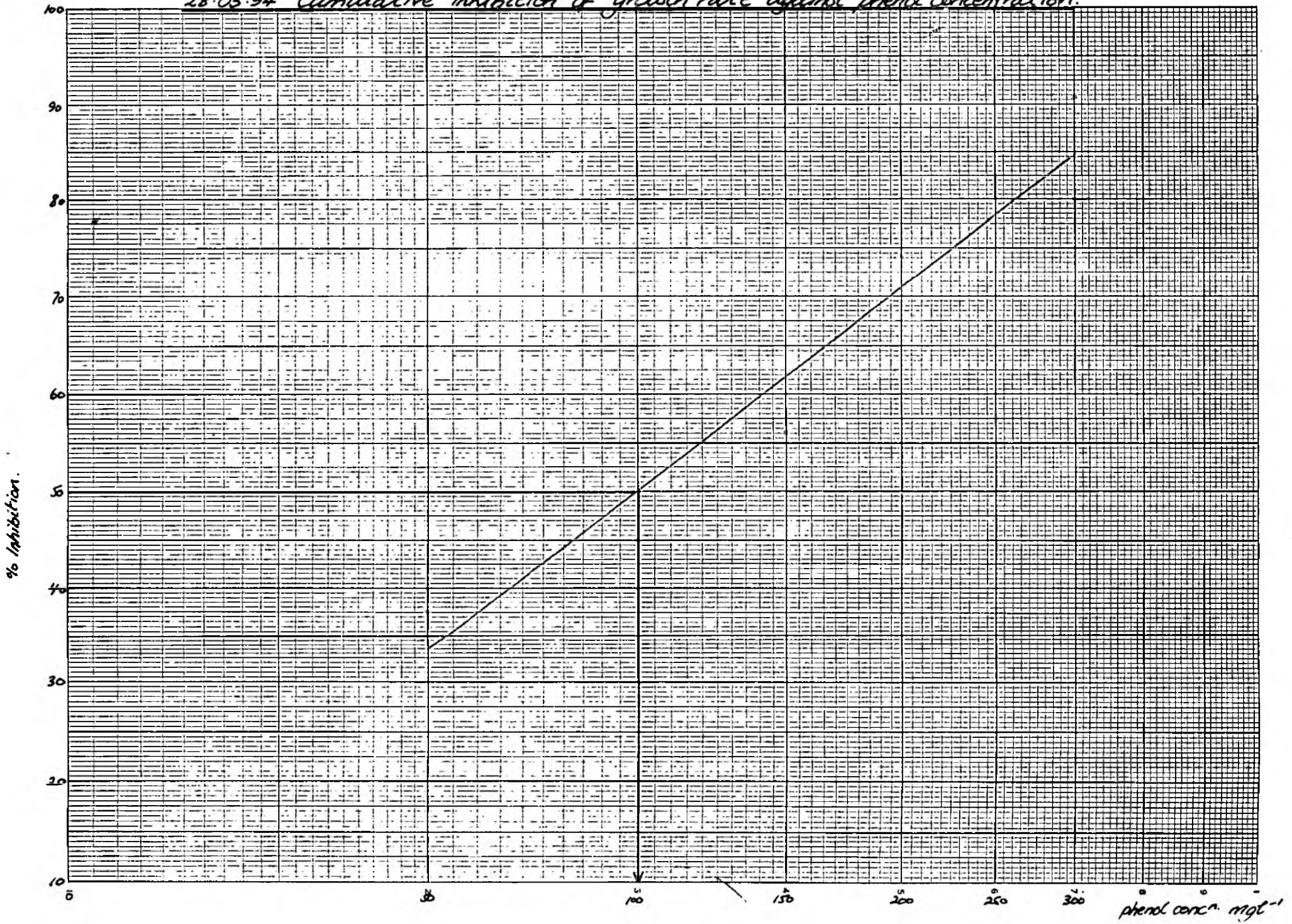
Dose response curve for Scenedesmus subspicatus.




21.03.94 Cumulative inhibition of growth rate against phenol concentration.
% inhibition.
0
10
20
30
40
50
60
70
80
90
0
50
100
150
200
250
300
phenol concn, mgl⁻¹

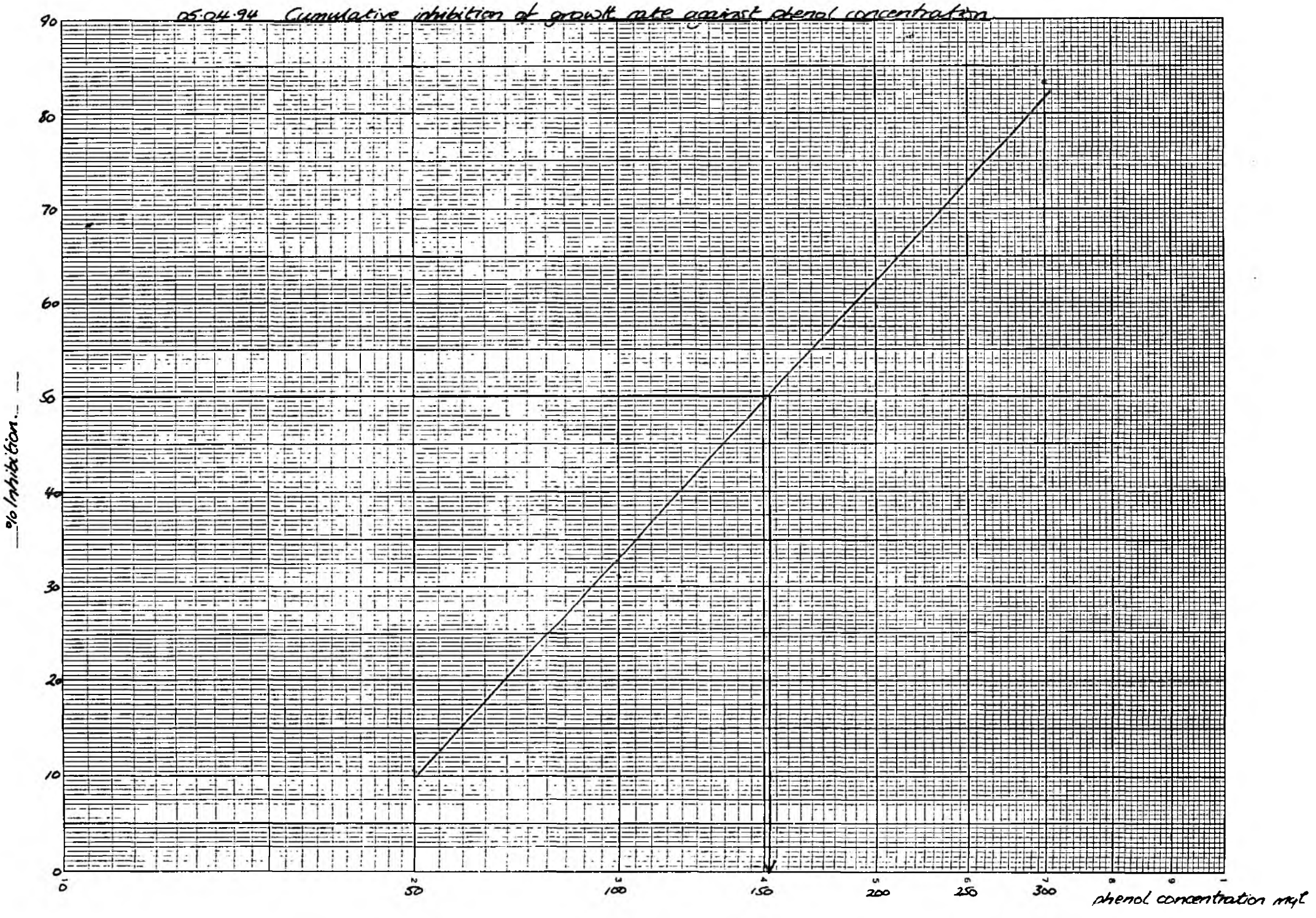
Chartwell Graph Data Ref. 5511 Log 1 Cycle x mm, ½ and 1 cm

28·03·94 Cumulative inhibition of growth rate against phenol concentration.



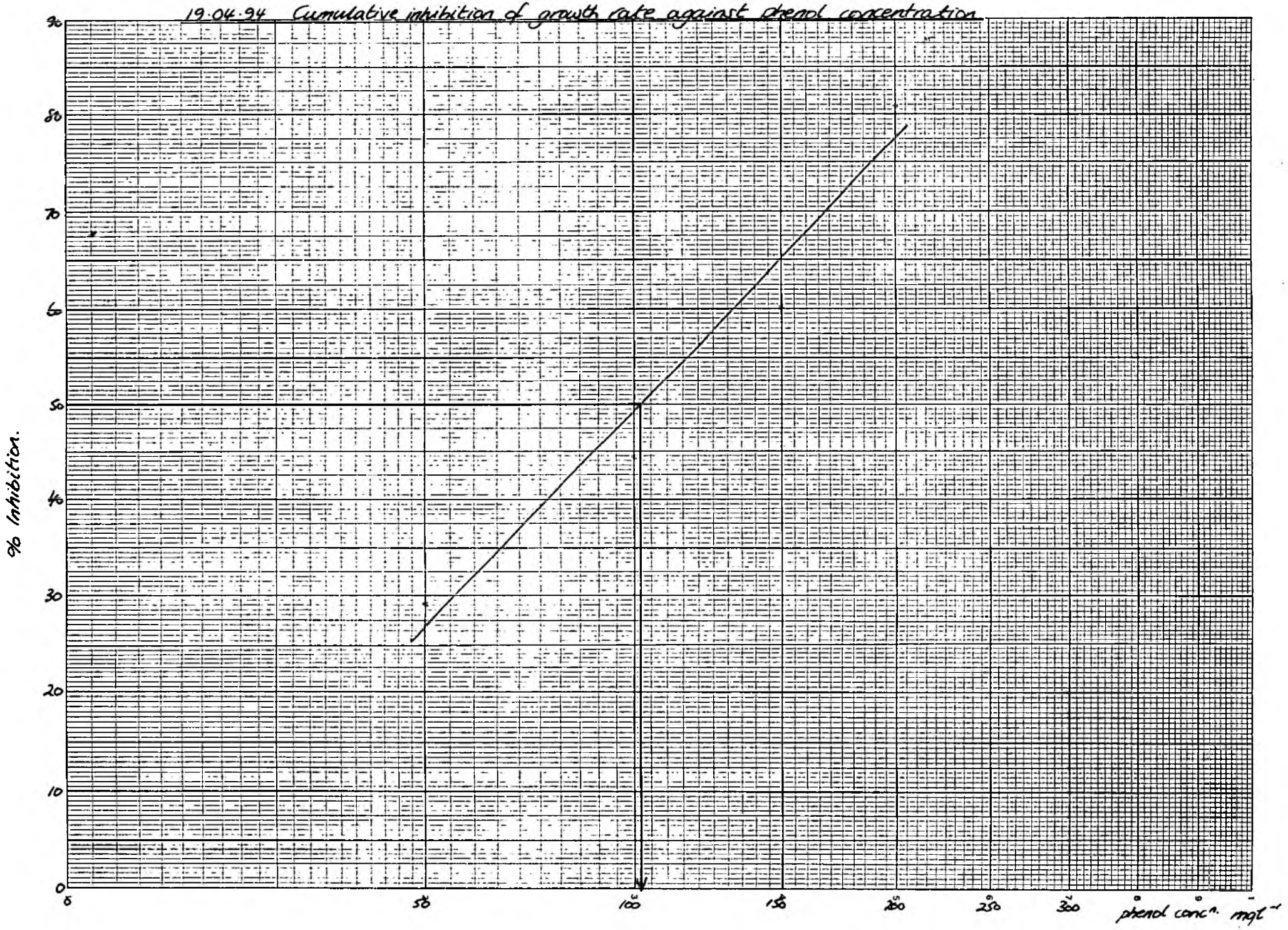
Chartwell Graph Data Ref. 5511 Log 1 Cycle x mm, ½ and 1 cm

05.04.94 Cumulative inhibition of growth rate against phenol concentration

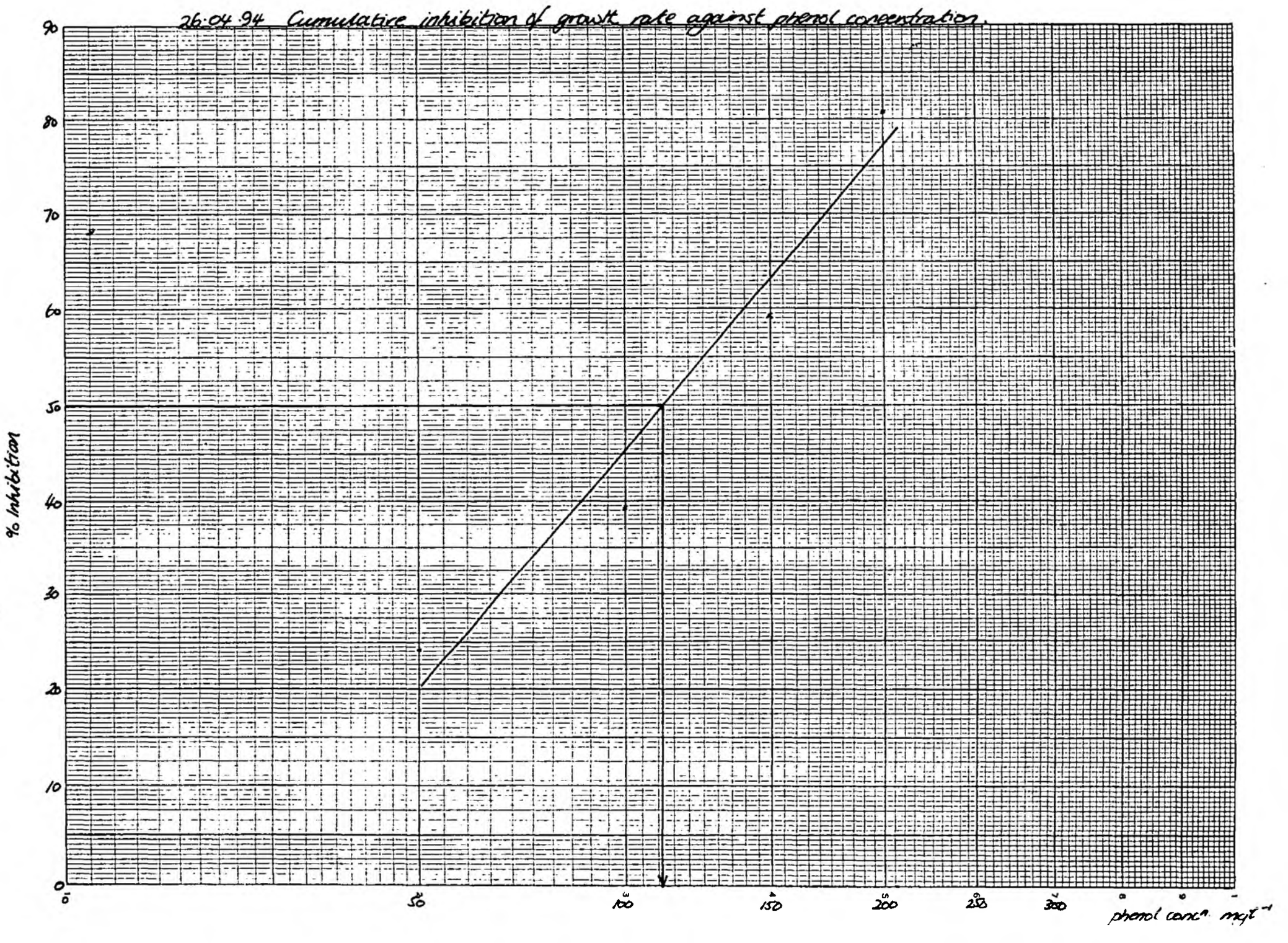


Chartwell Graph Data Ref. 5511 Log 1 Cycle x mm, ½ and 1 cm

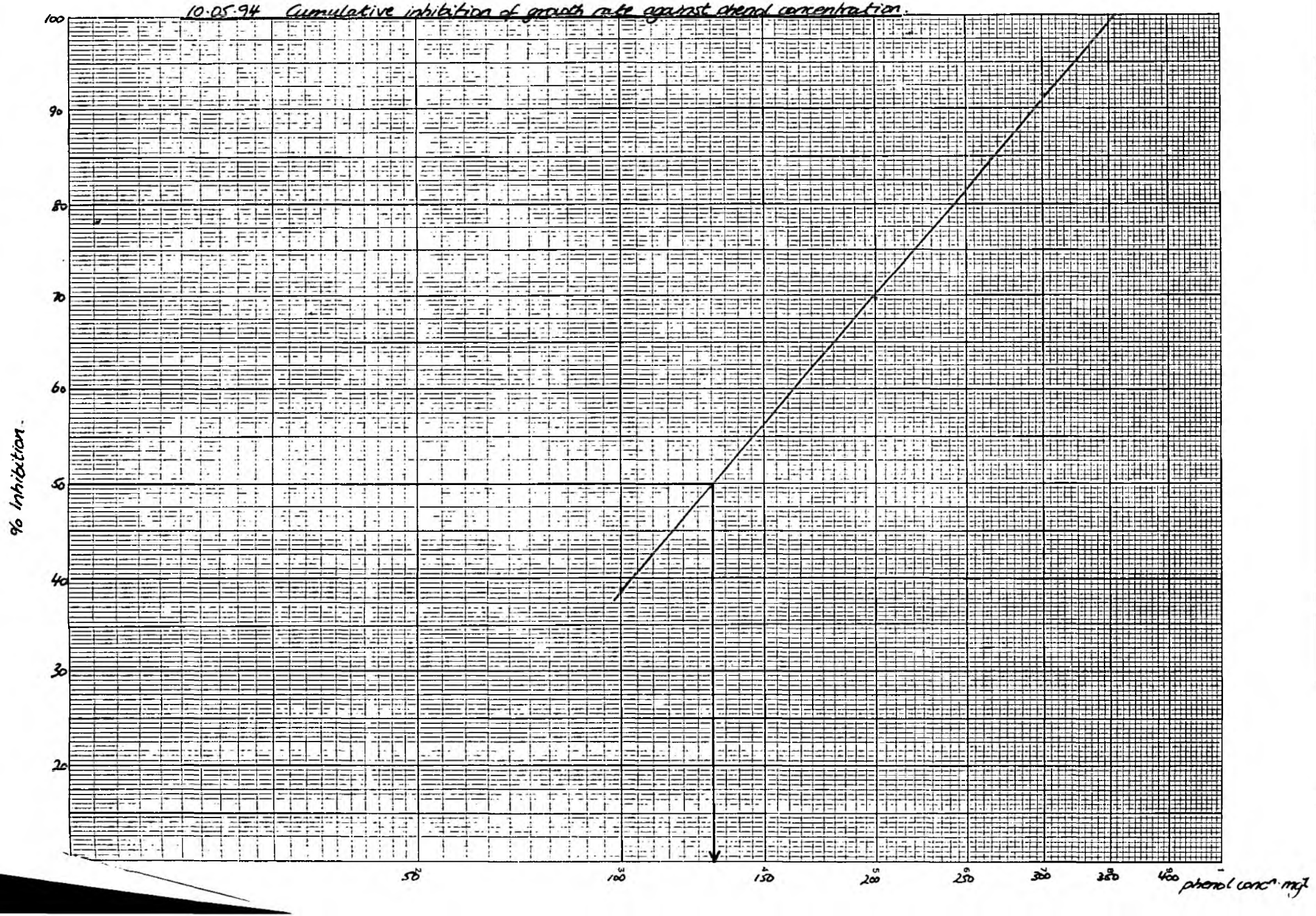
19.04.94 Cumulative inhibition of growth rate against phenol concentration



Chartwell Graph Data Ref. 5511 Log 1 Cycle x mm, ½ and 1 cm

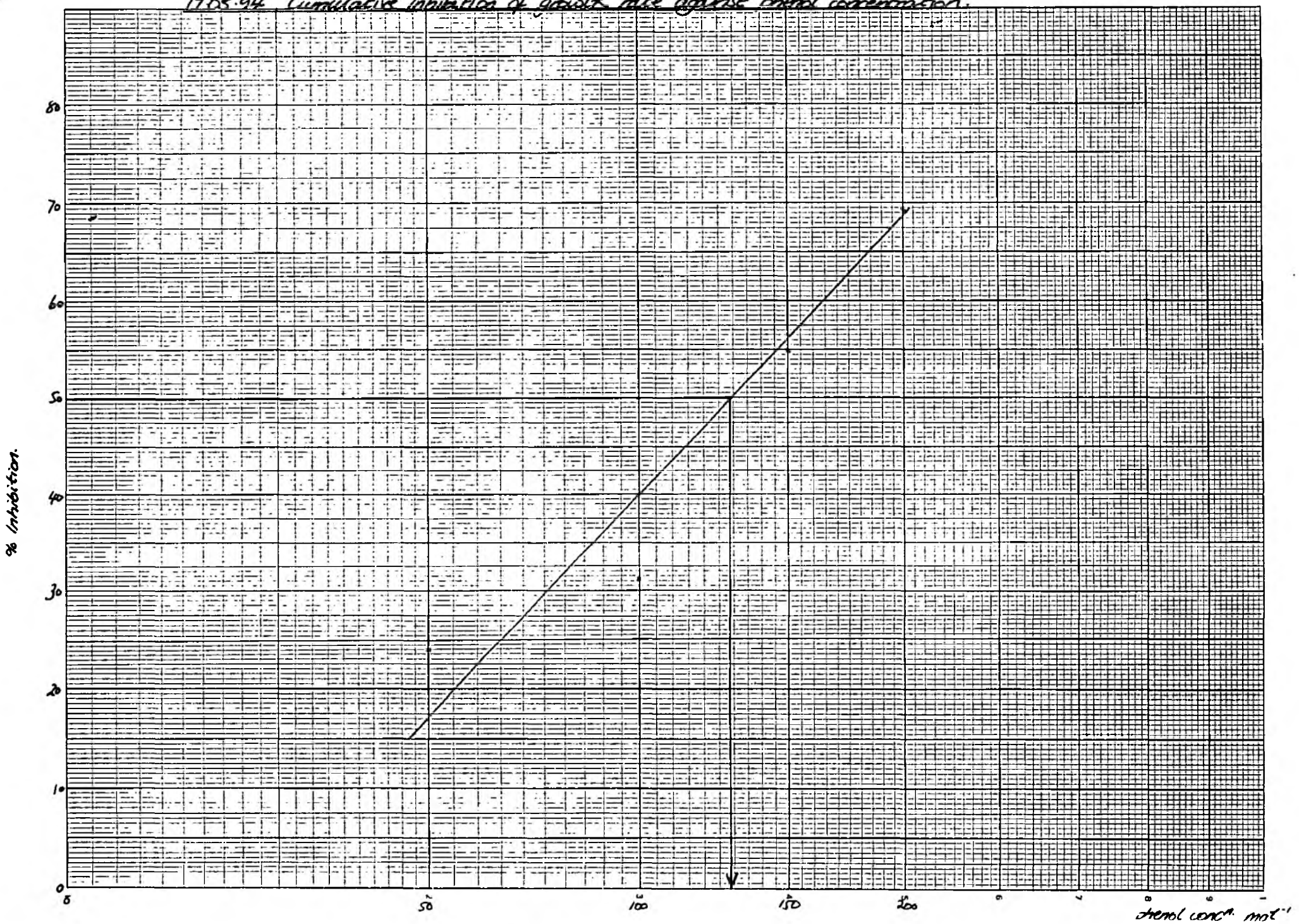


Chartwell Graph Data Ref. 5511 Log 1 Cycle x mm, ½ and 1 cm



Chartwell Graph Data Ref. 5511 Log 1 Cycle x mm, ½ and 1 cm

17.05.94 Cumulative inhibition of gouldt rate against phenol concentration.

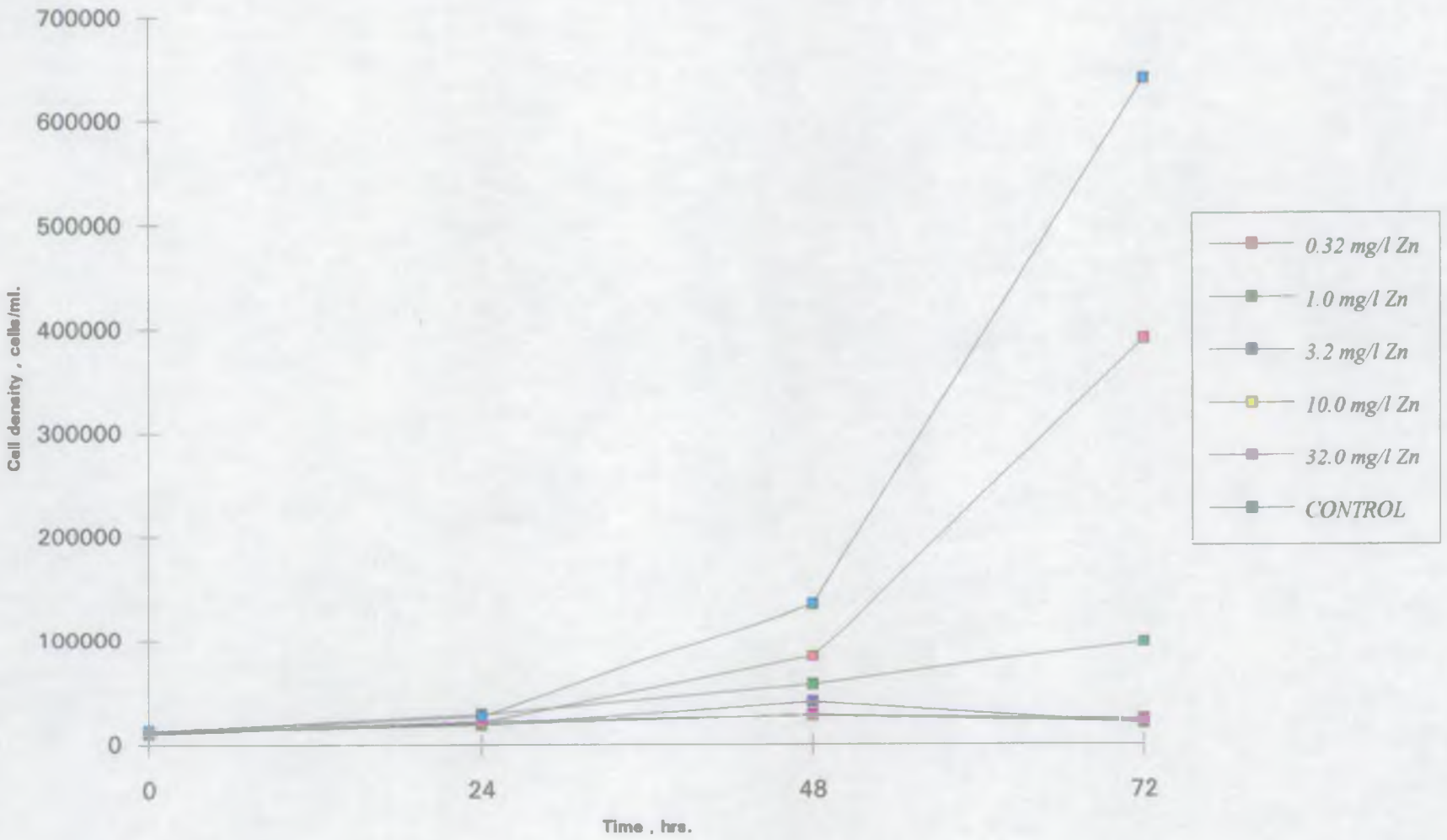


Section B: Individual test results for zinc:

NB/ no graphs appear for the first two tests due to the poor results.

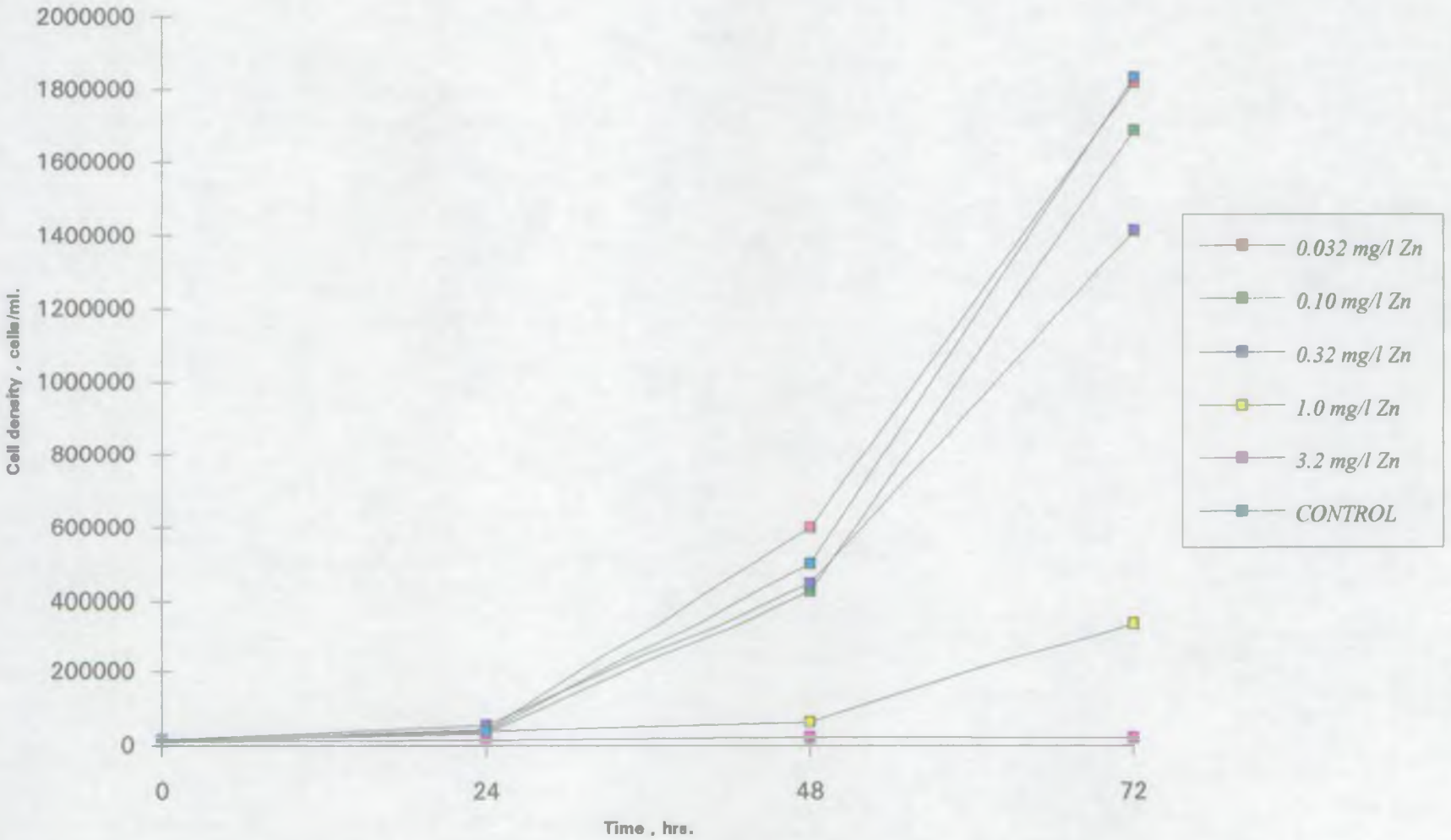
Test date: 10.05.94 **Test substance: Zinc**

Dose response curve for Scenedesmus subspicatus.



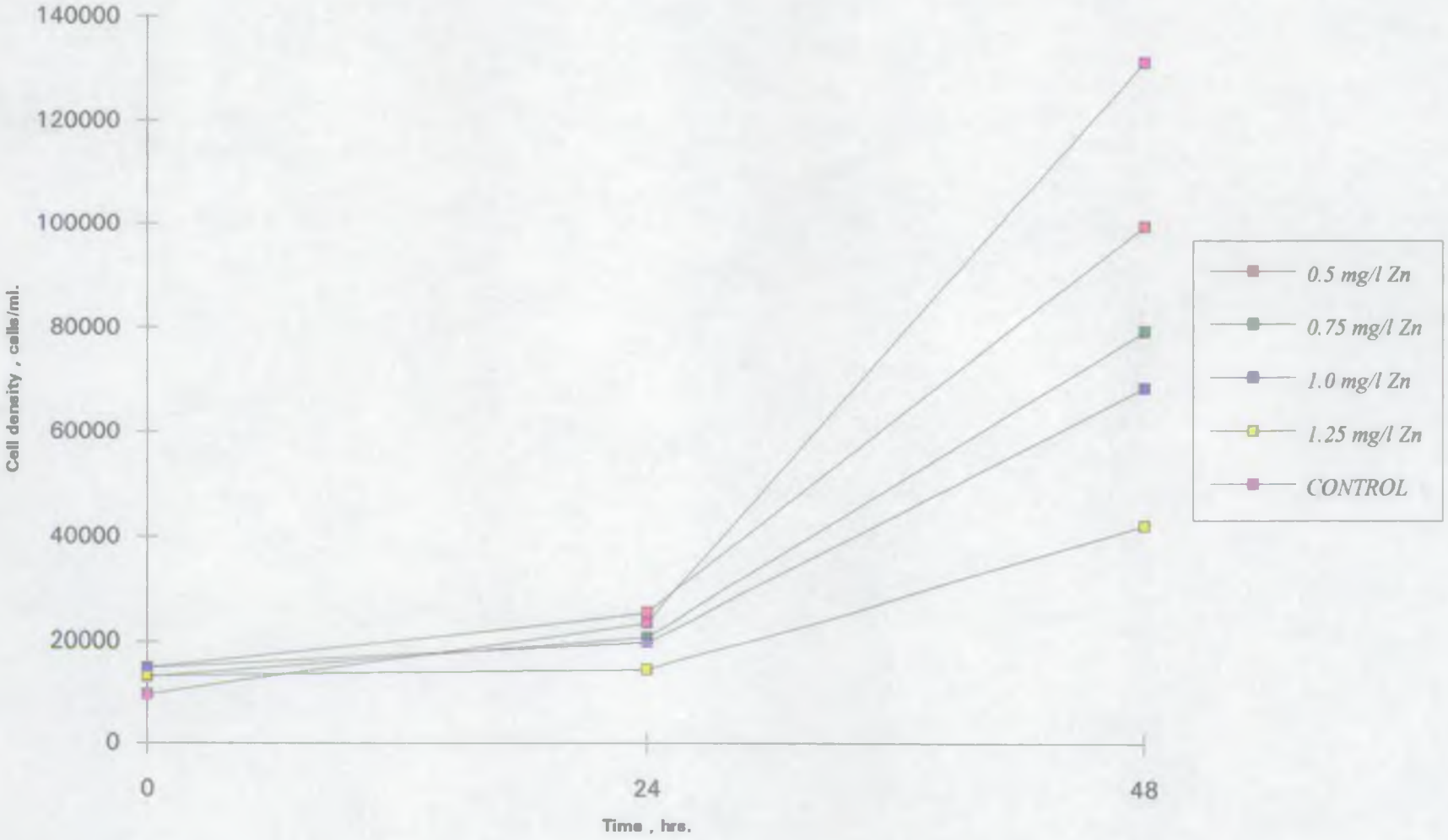
Test date: 17.05.94 Test substance: Zinc

Dose response curve for Scenedesmus subspicatus.

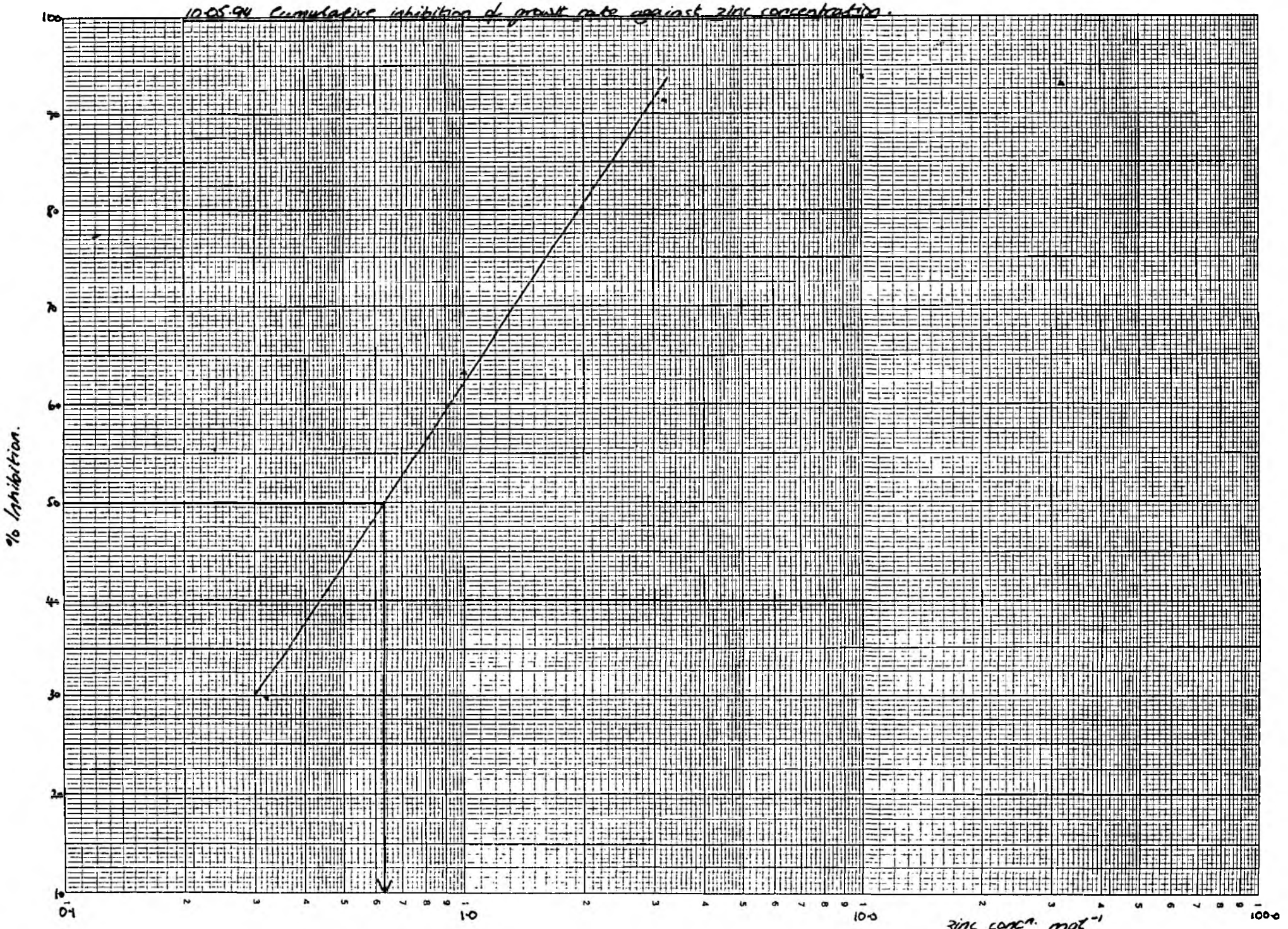


Test date: 22.06.94 Test substance: Zinc

Dose response curve of Scenedesmus subspicatus with zinc.

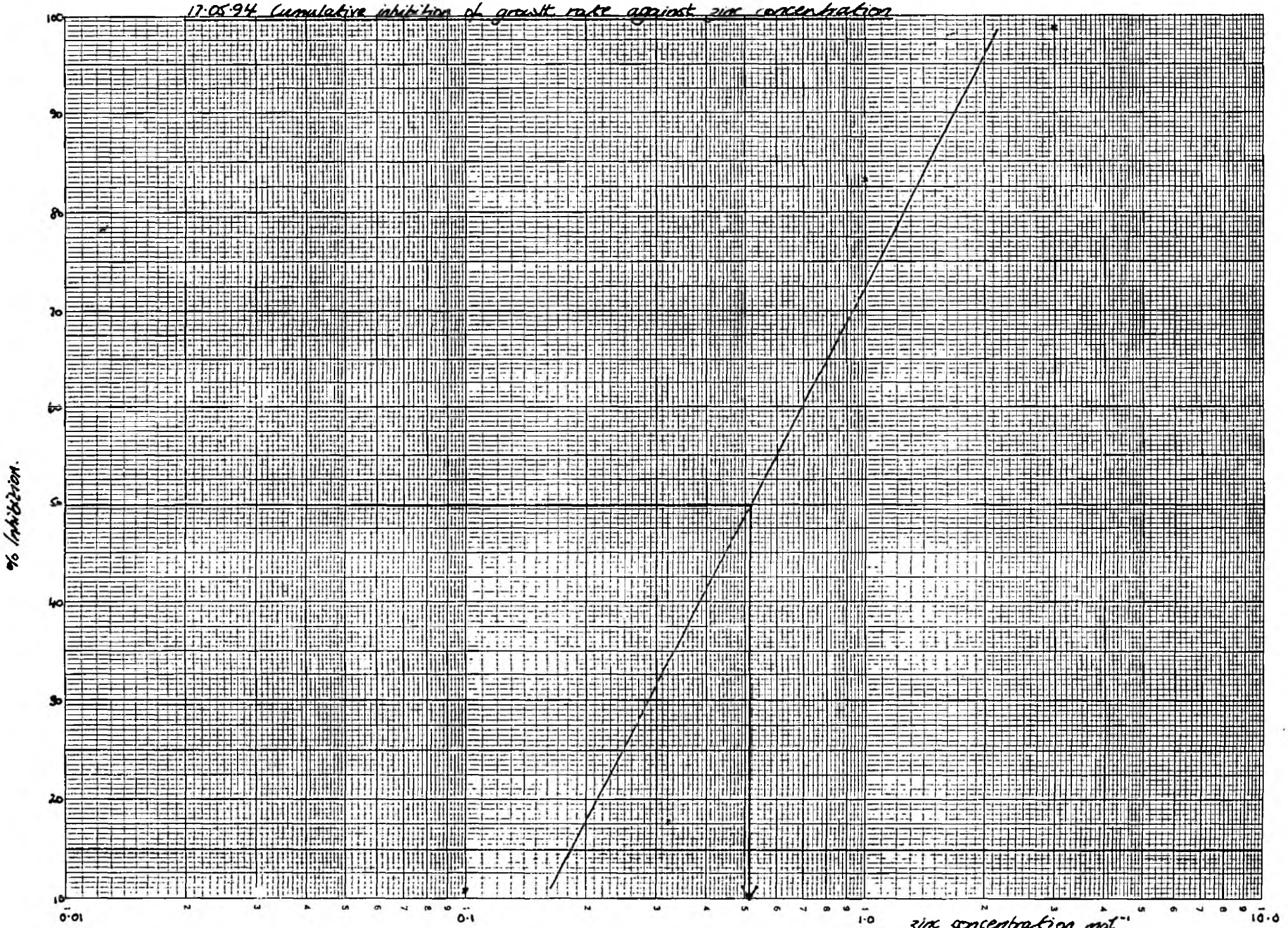


Chartwell Graph Data Ref. 5531 Log 3 Cycles x mm, ½ and 1 cm



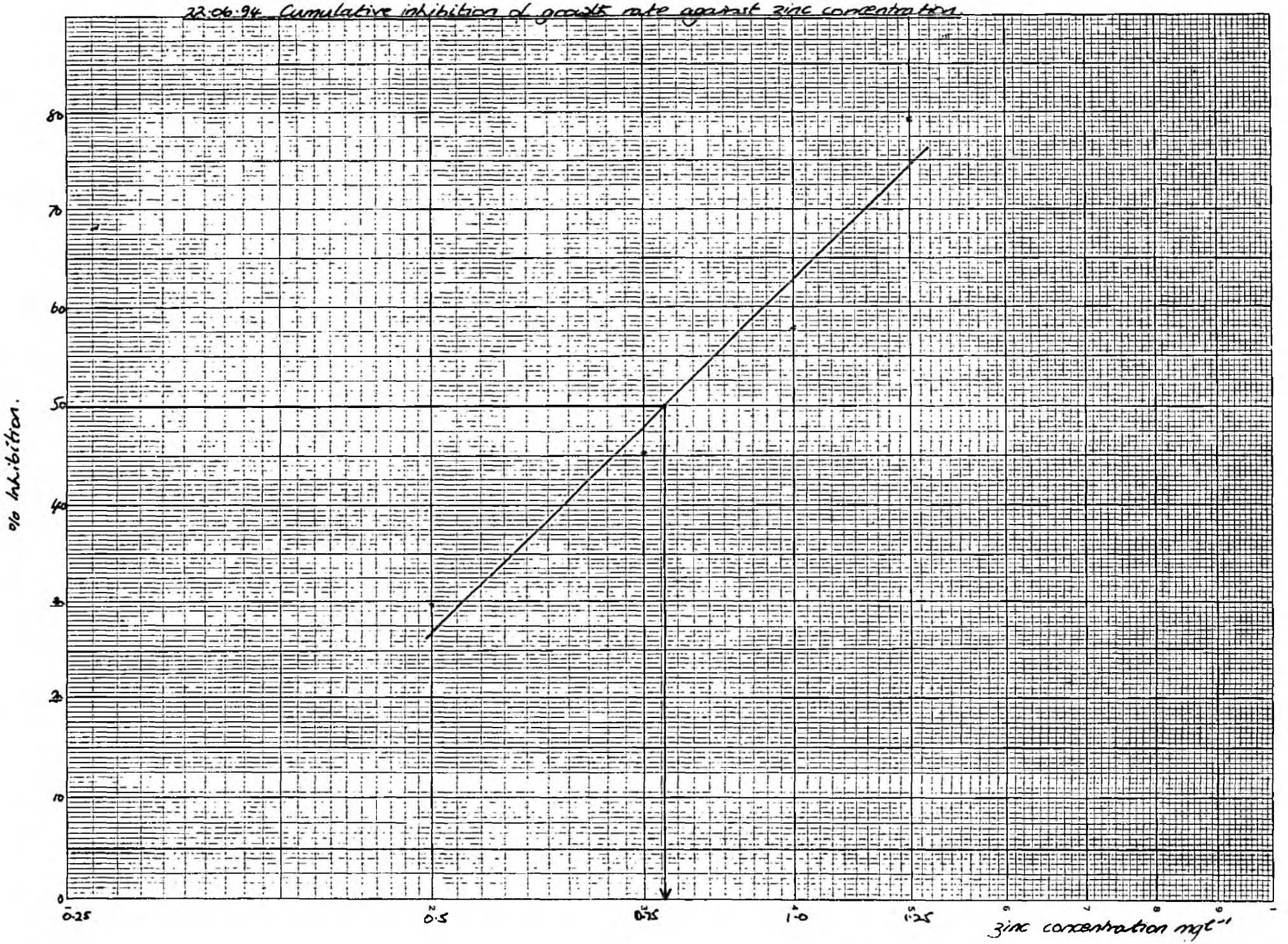
Chartwell Graph Data Ref. 5531 Log 3 Cycles x mm, ½ and 1 cm

17:05:94 Cumulative inhibition of growth rate against zinc concentration



Chartwell Graph Data Ref. 5511 Log 1 Cycle x mm, ½ and 1 cm

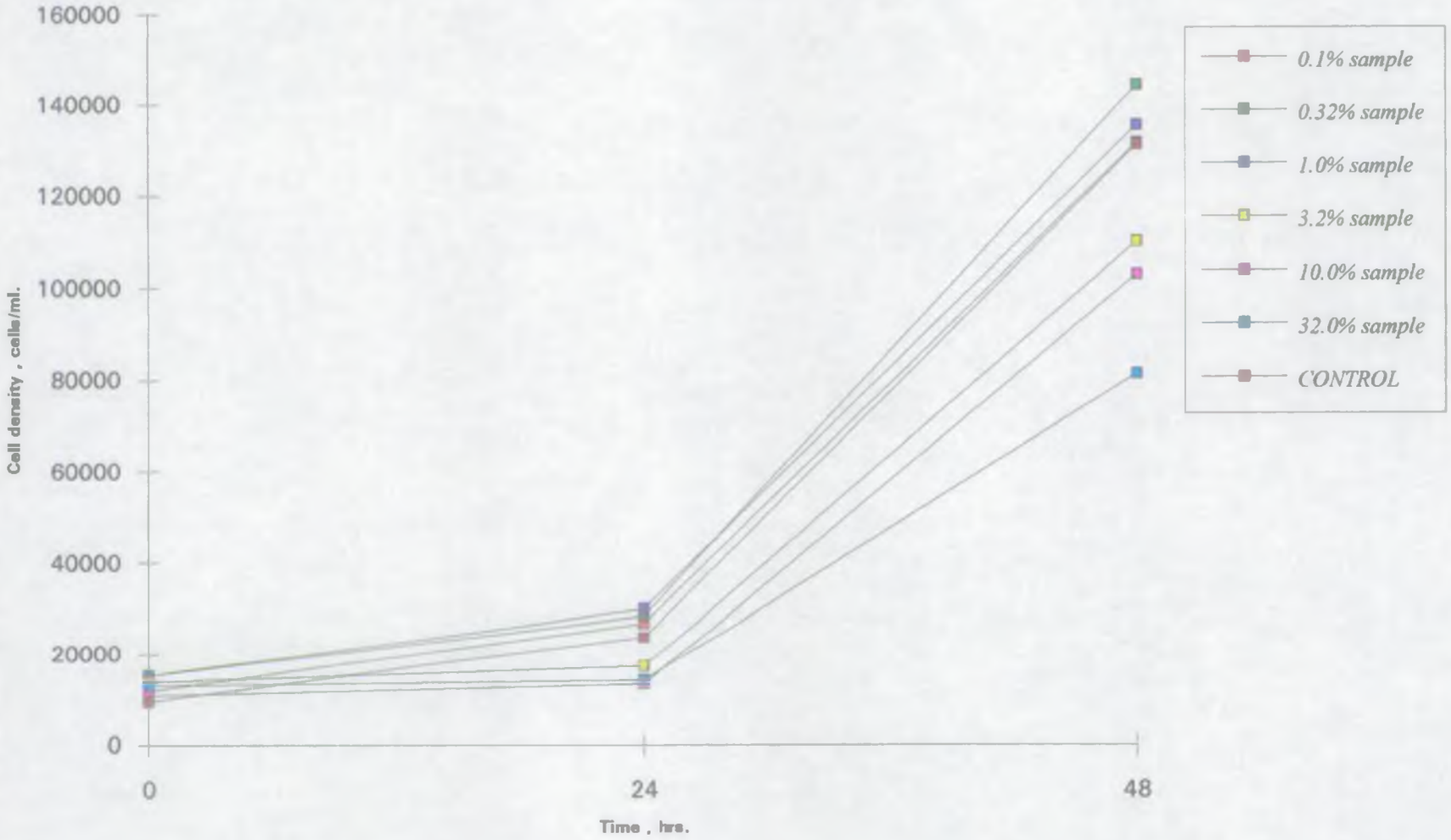
22.06.94 Cumulative inhibition of growth rate against zinc concentration.



Section C: Test results for R.Medway sample (receiving water) tested on 22.06.94 :

Test date: 22.06.94 Test substance: Receiving water

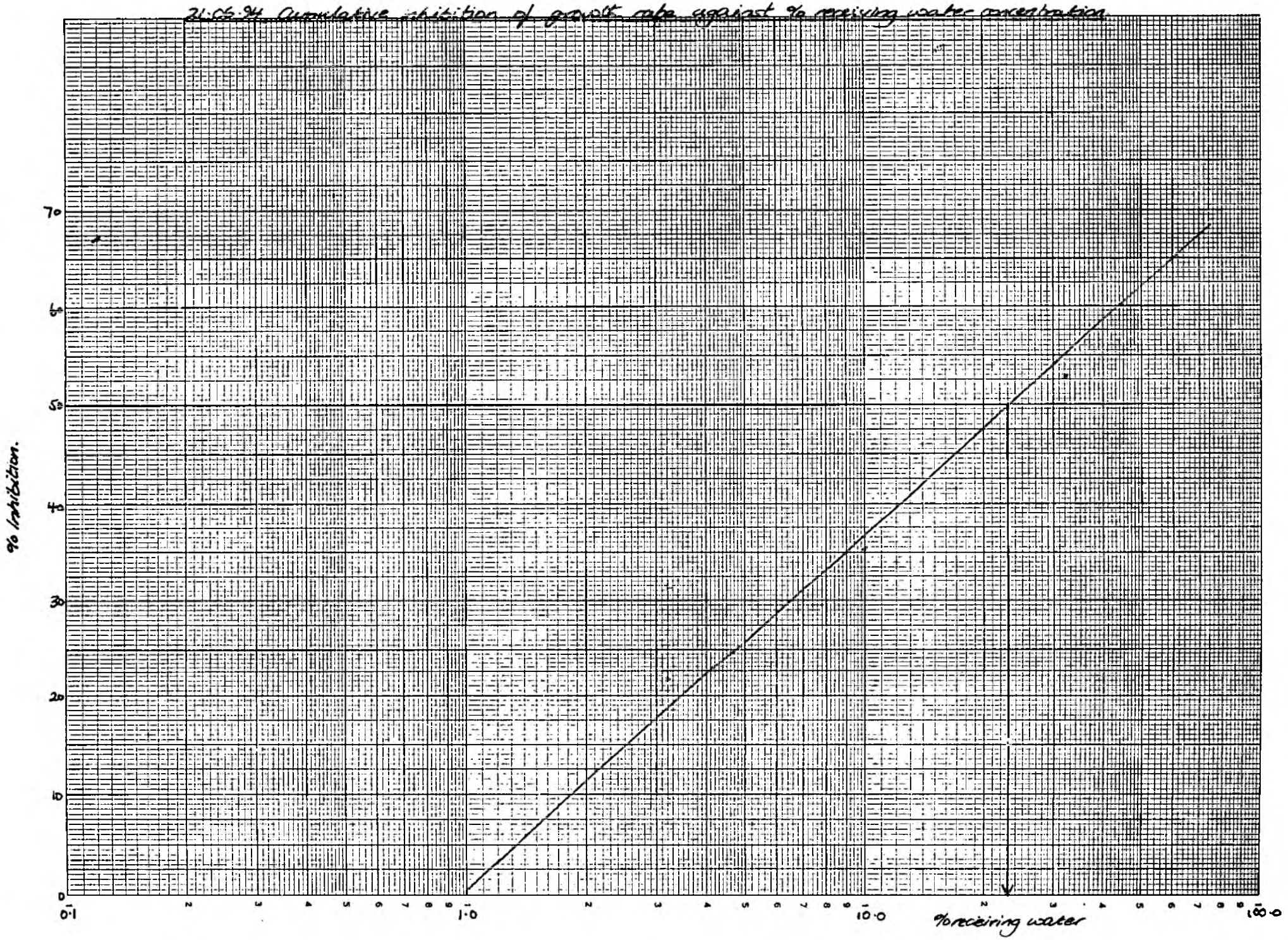
Dose response curve of Scenedesmus subspicatus.



Graph Data Ref. 5531

Log 3 Cycles x mm, ½ and 1 cm

21.06.94 Cumulative inhibition of growth rate against % receiving water concentration



Section D:

Table 6. Treatment of phenol results to produce control chart on page 14.

Table 7. Treatment of zinc results to produce control chart on page 18.

Table 6. Phenol data.

		Conc.								
		phenol	21.03.94	28.03.94	05.04.94	19.04.94	26.04.94	10.05.94	17.05.94	
		(mg/l)								
		50	29.4	37.5	10.6	29.1	24.1	-	24	
		100	49.3	45	31.1	44.4	39.3	38.7	31.2	
		150	-	56.1	-	60.3	59.1	-	55	
		200	74.1	74.1	59.6	80.9	80.7	69.8	69.4	
		300	86.2	90.8	83.4	-	-	91.4	-	
		400	-	-	-	-	-	>10	-	
	Control									
	growth rat	e (u/day)	1.689	1.388	1.293	1.566	1.285	1.303	1.625	
	72hr IC50	(mg/l)	104	100	152	102	111	130	128	
	Summary	*statistics*		Test date	72hr IC50	Mean	+ 2 SD	- 2 SD	+ 3 SD	- 3 SD
				21.03.94	104	118	156.66	79.63	175.91	60.37
	Mean	118.1429		28.03.94	100	118	156.66	79.63	175.91	60.37
	S' Error	7.278241		05.04.94	152	118	156.66	79.63	175.91	60.37
	Median	111		19.04.94	102	118	156.66	79.63	175.91	60.37
	S' Dev	19.25642		26.04.94	111	118	156.66	79.63	175.91	60.37
	Variance	370.8095		10.05.94	130	118	156.66	79.63	175.91	60.37
	Skewness	0.917047		17.05.94	128	118	156.66	79.63	175.91	60.37
	Range	52								
	Minimum	100								
	Maximum	152								
	Sum	827								
	Count	7								

Table 7. Zinc data.

				Conc.zinc	10.05.94	17.05.94	22.06.94		
				(mg/l)					
				0.1		10.8	-		
				0.32	29.9	17.7	-		
				0.5			29.7		
Summary	*statistics*			0.75			45.2		
				1	76.8	83.1	57.8		
Mean	0.65			1.25		98.8	79.3		
S' Error	0.081445			3.2	91.4		-		
Median	0.63			10	93.7		-		
S' Dev	0.141067			32	93		-		
Variance	0.0199		Control						
Skewness	0.625169		growth rat	e (u/day)	1.303	1.625	1.324		
Range	0.28		72hr-IC50	(mg/l)	0.63	0.52	0.8		
Minimum	0.52								
Maximum	0.8								
Sum	1.95								
Count	3		Test date	72hr IC50	Mean	+ 2 SD	- 2 SD	+ 3 SD	- 3 SD
			10.05.94	0.63	0.65	0.93	0.37	1.07	0.23
			17.05.94	0.52	0.65	0.93	0.37	1.07	0.23
			22.06.94	0.8	0.65	0.93	0.37	1.07	0.23

Section E. Table 8. Analysis of variance of results from test dated 19.04.94

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Controls
REP 1	1026720	1536400	1478400	1341440	878240	1722080	1104880
REP 2	1332320	1867760	1290640	1704960	1065280	1276720	1386000
REP 3	1517360	863860	1772320	1507600	1793120	1545680	1075200

Anova: Single-Factor

Summary

Groups	*Reps*	*Sum*	*Average*	*Variance*
Sample 1	3	3876400	1292133	6.14E+10
Sample 2	3	4268020	1422673	2.62E+11
Sample 3	3	4541360	1513787	5.89E+10
Sample 4	3	4554000	1518000	3.31E+10
Sample 5	3	3736640	1245547	2.34E+11
Sample 6	3	4544480	1514827	5.03E+10
Controls	3	3566080	1188693	2.94E+10

ANOVA

Source of Variation

	SS	*df*	*MS*	*F*	*P-value*	*F crit*
B' Groups	3.57E+11	6	5.96E+10	0.572516	0.746037	2.847727
W'Groups	1.46E+12	14	1.04E+11			
Total	1.81E+12	20				

Section F:

Table 9. Settings used on Coulter Counter throughout the project period. The threshold setting (in μm) determines the upper and lower limits to cell size which the coulter counts.

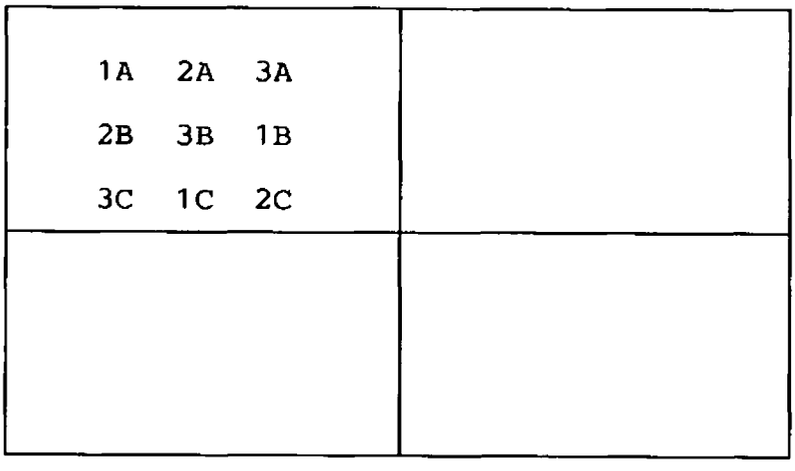
Figure 1. Diagram to show block design used to minimise the effects of uneven lighting across the incubator platform. The position of the first three concentrations in a series, including replicates (A, B and C) are shown. The block design was repeated in groups of three concentrations.

* *ISOTON*. Isoton II diluent is an azide-free balanced electrolyte solution containing 7.9 gl^{-1} sodium chloride. The solution is supplied by Coulter Electronics Ltd, for use in cell counting on the coulter counter.

Table 4. Coulter settings used during project period.

Calibration date	Tube orifice (µm)	Preset gain	Kd	Threshold (upper; µm)	Threshold (lower; µm)
(manometer volume 500 µl)					
23.11.93	70	2	8.543	12.58	3.474
15.04.94	70	2	8.517	12.60	3.901
(manometer volume 100 µl)					
22.06.94	70	1	10.60	12.60	3.936
11.07.94	70	1	10.82	12.59	3.931

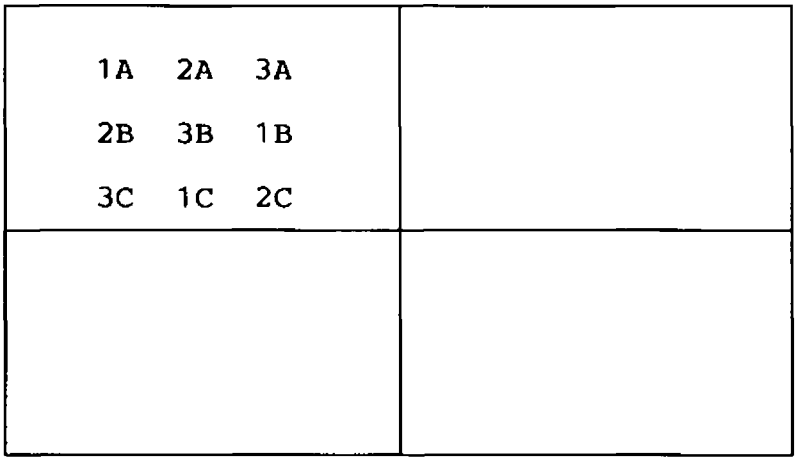
Figure 1. Diagram to show incubator platform.



3.8 Acknowledgements.

I would like to thank the everyone in the Biology Section for making my placement year so rewarding. I would especially like to thank Caroline Rutter and Tessa Crawshaw for their assistance with the ecotoxicology component.

Figure 1. Diagram to show incubator platform.



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