I.C.W.B.-C.I.P.S.

MATHEMATICAL MODEL OF
POLLUTION IN THE NORTH SEA

TECHNICAL REPORT
1973/PHYSIOL.-SYNTHESE 02

THE INFLUENCE OF INORGANIC AND ORGANIC POLLUTANTS ON THE RATE OF REPRODUCTION OF A MARINE HYPOTRICHOUS CILIATE : EUPLOTES VANNUS MULLER.

by

G. PERSOONE and G. UYTTERSPROT

State University of Ghent
Laboratory for Biological
Research in Environmental
Pollution

The influence of inorganic and organic pollutants on the rate of reproduction of a marine hypotrichous ciliate : Euplotes vannus Muller

INTRODUCTION

"Although the concentrations of pesticides and heavy metals in potential seafood is currently attracting widespread attention, the effects of pollution on lower trophic levels in the sea remain almost unknown. In the sediment ecosystem pollution could have far-reaching consequences at high trophic levels through for example alteration of the composition of important species at low trophic levels in food webs, or by irradication of organisms fundamental to the breakdown processes of the carbon, nitrogen and sulphur cycles."
(GRAY and VENTILLA, 1971)

Although some literature concerning the sensitivity of benthic marine invertebrates (especially macrofauna) with respect to inorganic and organic pollutants becomes slowly available, we only could find a few publications dealing with bioassays on marine ciliates.
FENCHEL (1967), however, stated :
"The biomass of marine ciliates in some types of sediments is of the same order or sometimes larger than the biomass of the micrometazoans (nematodes, turbellarians, gastrotrichs, etc...)" and COOLEY and KELTNER (1969) even stated that :
"The abundance of ciliates in aquatic ecosystems and their importance as nutrient regenerators and as food for other organisms are such that any significant increase or decrease in their population density should be reflected at other trophic levels."

In order to somewhat complete our very fragmentary knowledge concerning the influence of pollutants on this very important group of organisms, a series of experiments were conducted on the marine hypotrichous ciliate "Euplotes vannus" MULLER.
In previous experiments on this ubiquitous species which can be regarded as representative for marine sand-mud sediments, we attempted to establish the appropriate culturing parameters with respect to temperature, salinity and nutrition (PERSOONE and DEPLAECIE, 1972).
The pollutants investigated are those cited by the chemists working on the project "Mathematical Model of the North Sea", as regularly appearing in relatively high concentrations in the water and sediments at the 25 sampling stations of the investigated area of the North Sea.
It concerns the metal ions : lead, copper, mercury, zinc and cadmium; as well as the pesticides DDT, DDE, Lindane (hexachlorocyclohexane), hexachlorobenzene and heptachlor. Finally, the toxicity of arochlor 1254 as an example of a polychlorinated biphenyl (PCB) was tested.

MATERIALS AND METHODS

a. Test organisms :

The Euplotes vannus used in the tests were isolated from "fouling" material formed on substrates submerged in the Ostend harbour. Since several specimens were brought together after isolation, the used stock culture was not clonal.

b. Feeding :

The test organisms were cultured on a yeast - seawater suspension (yeast in powder form : Fleischmann's Dry yeast). The stock solution was made up of 1 g yeast per 100 ml distilled water. The feeding medium itself consisted of 1 ml stock solution per 100 ml seawater.

c. Seawater :

Artificial seawater with a salinity of 35 °/₀₀ made up according to the formula of DIETRICH and KALLE (1963) was always used.

d. Temperature :

The culturing vessels were incubated in an oven at 28°C.

e. Culturing vessels :

Polystyreen transparent plates with 8 round depressions (cells) (KARTELL "kuvetten" TS 357) were used. The cells have a flat bottom and a diameter of 15 mm. They each can contain 0,5 ml liquid. To reduce evaporation of the medium, the plates were put in Petri dishes on humid filter paper after inoculation.

f. Preparation of the dilutions of the various pollutants :

1. Metal ions :

In order to disrupt the normal ionic balance of seawater as little as possible, the chloride salt of all tested metal ions was used.

$PbCl_2 \cdot 2H_2O$ for Pb^{++}
$CuCl_2$ for Cu^{++}
$HgCl_2$ for Hg^{++}
$ZnCl_2$ for Zn^{++}
$CdCl_2 \cdot 2{,}5H_2O$ for Cd^{++}

Since several of these salts are hardly soluble in seawater, at least at higher concentrations, they were first dissolved in 250 ml distilled water which was then added to 750 ml seawater, itself derived from 1 l seawater with a salinity of 35 °/₀₀ which was previously evaporated to 750 ml.

2. Pesticides and PCB

Since none of these substances are easily soluble in water, they were first dissolved in a "carrier", which in this case was acetone.

To this solution acetone was further added to obtain a dilution series so that 1 ml of the respective dilutions added to 1 l feeding medium, (seawater + yeast) resulted in the required concentration of pollutant. In this manner, the following concentrations were prepared and subsequently tested :
10 ppm, 1 ppm, 100 ppb and 10 ppb.
As control, solutions were used to which 1 ml acetone per 1 seawater had been added.

g. Inoculation :

From the stock culture in exponential phase, the test organisms are sucked out one by one with a micropipette and transferred to the cells (already filled with liquid) of the culturing plates at a ratio of 1 individual per cell. For each concentration of each tested pollutant, 20 replications were inoculated.

h. Duration of the experiments :

After 48 hours all test organisms are fixated by adding 0,1 ml Bouin to each culture cell. The number of organisms is then counted under a dissection microscope and the number of divisions computed according to the method of GENERMONT (1969) :

$2^x < n < 2^{x+1}$ in which n is the number of ciliates after 48 hours and x the number of generations. Usually n is larger than 2^x, meaning that some specimens have already undergone the x+1 division. In our computations we only took this x+1 value into account.

RESULTS (Figures 1 through 11)

During our previous investigations on this species (PERSOONE and DEPLAECIE, op. cit.) it was already found that the variability among the different replications can be rather larger. In the controls of most tests the 7th generation was reached at least in some of the replications (more than 64 organisms and less than 128). In some other replications, on the contrary, the ciliates were only at the 4th division (8 to 16 organisms). In comparing the average number of generations of the controls in the 20 replications of each of the 11 tests performed, it appears that the minimum is 4,9, the maximum 7. The causes of these differences can be of various origins and were already discussed in our previous work.
The toxicity of the tested substances was calculated as the procentual inhibition, meaning the decrease in the number of divisions.

For each dilution the average number of generations in the 20 replications was calculated and compared with the average number of generations in the controls. The ratio of these two figures, expressed in % and substracted from 100, gives the procentual inhibition.
Mathematically:

$$\% \text{ inhibition} = 100 - \left(\frac{\text{average number generations + pollutant}}{\text{average number generations control}} \times 100 \right)$$

A. Heavy metals

a. Lead (Fig. 1)

Up to 100 ppb no influence was detected. At 1 ppm 16 % inhibition exists, at 10 ppm 29 %, and at 100 ppm no division occurs in any of the 20 replications and all the test organisms are dead.

b. Copper (Fig. 2)

Already at 1 ppm an inhibition of more than 40 % exists while the inhibition is total at 10 ppm.

c. Cadmium (Fig. 3)

At 10 ppm, 20 % inhibition occurs, and at 100 ppm all ciliates died.

d. Zinc

Inhibition of 10 and 100 % occurs at levels of 10 and 100 ppm respectively.

e. Mercury

Appeared to be the most toxic metal :
the 1 ppm level was lethal to all test organisms;
at 100 ppb, however, no inhibition existed.

B. Pesticides and PCB (Figures 6 through 11)

Contrary to the results obtained with most metal ions, the toxicity of the pesticides at a concentration of 10 ppm caused an inhibition only ranging from 3 to 10 % compared to the control. Although the performed t-tests show a statistically significant difference ($P<0,05$) between the average number of generations at the 10 ppm level and the control, we do not dare to give a mathematical interpretation of these inhibitions. Some of the lower concentrations tested, showed indeed up to a 10 % deviation from the control.
With Arochlor 1254 the inhibition was even 0 at 10 ppm. This agrees very well with our results obtained from experiments done during a stay at the Duke University Marine Laboratory at Beaufort, N.C., with this chemical on an american stock of Euplotes vannus.
Since at the commencement of this study the hypothesis was set for that the pesticide and PCB concentrations in the marine environment would hopefully never exceed the 10 ppm level, the 100 ppm dilution was not tested, contrary to the metal ions. We therefore can only guess if this concentration would affect the ciliates in some way or another.

DISCUSSION

In Fig. 12 all the procentual inhibitions of the 11 tested substances were represented, starting from the 100 ppb level. From the graph it appears that most metal ions are much more toxic to the test organisms used than the organic pollutants.

Among the heavy metals mercury is the most toxic product. It is remarkable that for this substance the 1 ppm level inhibits totally while the 100 ppb level seems absolutely not toxic.

Copper comes at the second place with 50 % inhibition at the 1 ppm level and 100 % at 10 ppm; lead, cadmium and zinc somewhat inhibit the divisions at 1 and 10 ppm but total inhibition only occurs at 100 ppm.

As already mentioned, was the inhibition lower than or equal to 10 % at a concentration of 10 ppm of the tested organic substances.

The only study we found concerning the influence of heavy metals on a marine ciliate is the one of GRAY and VENTILLA (1971).

These authors working with a marine pelagic ciliate : Cristigera sp. found that $HgCl_2$ provoked already a 100 % mortality at 20 ppb, while 5 ppb led already to a reduction growth rate of 12 %.

Moreover, 250 ppb zinc sulphate gave a 13 % inhibition; 30 ppb lead nitrate was toxic for 12 %, but at 300 ppb this substance was not yet 100 % toxic. The authors added that by mixing these salts, the toxicity increased considerably due to the synergetic effect of the pollutants.

As far as the sequential order of toxicity is concerned the results of GRAY and VENTILLA on Cristigera sp. agree with our observations on Euplotes vannus, namely, the lowest toxicity for zinc, followed by lead, and the highest for mercury.

For the 100 % inhibition, we only can compare the mercury values (for zinc and lead the authors give no data).
From these findings it seems that the sensitivity of Cristigera to mercury is much higher than that of Euplotes vannus : our results show indeed a total inhibition somewhere between 100 ppb and 1 ppm, whereas GRAY and VENTILLA found a 100 % mortality at 20 ppb.
The results obtained with the other two metal ions point in the same direction : the authors mentioned above, found here also inhibitions at considerably lower concentrations than in our experiments with Euplotes.
It is indeed very difficult to carry out a close comparison between the experiments of GRAY and VENTILLA and ours; the authors worked in completely different circumstances with a completely different methodology.
Starting from cultures in the exponential phase, the reduction in growth is measured every hour for 5 to 7 minutes, by counting the number of organisms per unit volume (automatic counting with the Coulter-counter). The tests were carried out in duplicate + 1 control and the results computed and plotted as a change of the growth rate (increase in population) $\Delta \frac{Ntn}{Nto}$.

Recently, a very interesting contribution to the influence of toxicants on freshwater protists was published by RUTHVEN and CAIRNS (1973). These authors determined the lethal concentration and the tolerated concentration for various rhizopods, flagellates and ciliates, for different metal ions (in the form of various salts) of inorganic acids and phenol.
The methodology used differs very strongly from the one we used : the "lethal concentration" is the lowest concentration at which all organisms die after 10 minutes (microscopical observation) ; the "tolerated concentration" is the highest concentration at which some test organisms still live after 3 hours.

For three of the metals we also tested, RUTHVEN and CAIRNS found the following results with the ciliates (see table).

in ppm		Tetrahymena sp.	Paramecium caudatum	P. multimicro-nucleatum	Stentor coeru-lius	Blepharisma sp.
Cu^{++} ($CuSO_4 . 5H_2O$)	A	$\frac{10}{0,32}$	$\frac{10}{1,35}$	$\frac{0,1}{0,032}$	1.0	$\frac{3,2}{0,1}$
	B		$\frac{}{1,35}$	$\frac{1.0}{0.24}$		$\frac{3,2}{0,18}$
	C					$\frac{1,8}{0,32}$
Pb^{++} ($Pb(NO_3)_2$)		$\frac{100}{24}$		$\frac{56}{24}$		$\frac{100}{42}$
Zn^{++} ($ZnSO_4 . 7H_2O$)	A	$\frac{5,6}{1,0}$	$\frac{32}{15,5}$	$\frac{10}{0,56}$	42	$\frac{100}{10}$
	B					$\frac{32}{5,4}$
	C					$\frac{56}{5,6}$

Numerator : lethal concentration

Denominator : tolerated concentration

Since it seems that these experiments were in no way standardized, it is extremely difficult to make any comparisons with our results. Concerning the very divergent figures obtained with the same toxicant in the various replications, the authors state :
"When a compound was tested more than once with a given species, variations in result probably were due to changes in the culture medium (i.e. pH, temperature, etc...) and test organisms being in a different growth phase. These factors plus exposure time, dissolved O_2 and CO_2 concentrations, other heavy metals, hardness, complex formation, species resistance, and adsorption on submerged objects are all known to modify heavy metal toxicity."
They admit indeed that these experiments are a "first approximation of response to a variety of toxicants".
From their results we can nevertheless conclude that copper showed the highest toxicity and that zinc seems to be considerably more poisonous than lead for freshwater ciliates.
The lethal concentrations which they noted are roughly of the same order of magnitude as those which we observed for Euplotes, namely 1-10 ppm for copper, 10-100 ppm for lead, and 10-100 ppm for zinc. From the "tolerated concentrations" given by RUTHVEN and CAIRNS it appears, however, that our Euplotes was much more resistant to copper and zinc as far as survival is concerned, than the freshwater ciliates used by these authors.
Another interesting finding in their experiments is the fact that "the relative sensitivity of protozoa to various toxicants will not always be the same, i.e. species X may be twice as tolerant to a toxicant as species Y but its relative sensitivity may be quite different for another toxicant."
Finally, several authors already examined the influence of mercury salts on Tetrahymena species, which are very well

known physiologically and biochemically.
TRASHER and ADAMS (1972) arrived at the conclusion that mercuric chloride ($HgCl_2$) is 20 times less toxic than organo-mercurials such as ethylmercuric chloride and phenylmercuric acetate. The latter are in turn a little less toxic than the methylated form of $HgCl_2$.
The concentrations which prolong the division rate a 100 % (thus double) are respectively : 4,34 ppm for $HgCl_2$; 0,196 ppm for ethyl-$HgCl_2$; 0,177 ppm for phenylmercuric acetate and 0,141 ppm for methyl-$HgCl_2$.

CARTER and CAMERON (1973) performed a classical TL_m on the same species and found a 50 % mortality with $HgCl_2$ at 3,12 ppm after 96 hours.
Proceeding from this result, TINGLE et al. (1973) investigated if lower concentrations of $HgCl_2$ (so called sublethal concentrations) could affect cell structures (cytotoxic effects).
They found indeed that 0,25 ppm of the toxicant caused "extensive but repairable sublethal damage", but that at 0,50 ppm "the damage persisted and accumulated with time up to 24 hours."
In the literature we could not find any figures for comparison, as far as the influence of organic pollutants on marine ciliates is concerned.
Many authors have, on the contrary, examined the toxicity of pesticides on freshwater ciliates.
GREGORY et al. (1969) investigated the influence of DDT and parathion on Paramecium bursaria and P. multimicronucleatum.
They determined that a pesticide concentration of 1 ppm, even after 7 days incubation, had absolutely no "adverse effects", although the pollutants seemed to be concentrated a hundred fold by the test organisms.
MORGAN (1972) even found that neither DDT, nor Arochlor 1248 seemed to have any influence on Tetrahymena vorax at concentrations as high as 100 ppm.

COOLEY and KELTNER (1969) found on the contrary, that T. pyriformis show a statistically significant reduction in growth rate at 10 ppm DDT.
COOLEY et al. (1973) noted that PCB's of the Arochlor - group (namely Arochlor 1248 and Arochlor 1260) started to be toxic around the 1 ppm level for T. pyriformis.
Previous results obtained by COOLEY et al. (1972) with Arochlor 1254 are rather strange. Arochlor 1254 of which the chlorine content is situated between that of Arochlor 1248 and 1260, would be 1000 times more toxic than the two previously mentioned Arochlors.

This last result is, in any case, totally opposite to our own findings with Arochlor 1254 on Euplotes vannus. The hypotrich seemed to be unsensitive to 10 ppm of this substance, which was indeed confirmed by our experiments with an american stock of Euplotes vannus (see section RESULTS).
Let us, for the sake of completeness, mention that the methodology of the authors cited, is very different of ours.
With Tetrahymena the population densities of the cultures are determined with a spectrophotometer at a certain wave length, subsequently the reduction of the growth rate compared to the control is calculated.
From all these results the soundness of the already mentioned citation by RUTHVEN and CAIRNS (op.cit.) appears again, namely, the highly divergent sensitivity of various species to a same toxicant.
Abstraction made from the high but dubious toxicity of Arochlor 1254 to T. pyriformis, mentioned by COOLEY et al., it appears nevertheless that the pesticides tested are generally less toxic than the heavy metal ions.
It makes little sense to compare the determined toxicity levels with those of phytoplanktonts, invertebrates or vertebrates. The literature data with respect to this fact, already prove that the sensitivity is indeed very

species-bound and that it can vary in orders of magnitude.

CONCLUSION

Looking at the results obtained by the chemists with respect to the maximal concentrations of pollutants determined in the water and sediments of the North Sea and the Scheldt estuary, we can make the following considerations :

TABLE 2

I. HEAVY METALS

	North Sea water (coast)	Scheldt water	North Sea sediment	Scheldt sediment
Zn	100 ppb	430 ppb	271 ppm	926
Cd	5 ppb	15 ppb	?	?
Pb	58 ppb	35 ppb	280 ppm	185
Cu	59 ppb	130 ppb	58 ppm	221
Hg	0,8 ppb	?	1,24 ppm	?

II. PESTICIDES

a. In water :

Concentrations usually very low to undetectable (0,01 ppb), exceptionally up to 1,5 ppb.

b. In sediments :

1 ppb up to a few ppb

III. PCB

a. In water :

?

b. In sediments :

up to 30 ppb

The values found in the coastal water lie far below the sensitivity threshold of the test organisms in question. The high concentration of zinc ions which is sometimes encountered in the Scheldt water, approaches, however, the inhibition threshold of the (although very resistant) Euplotes.

The situation is much more alarming as far as the concentrations of heavy metals tied up in the sediments is concerned.

The metal ions involved, are indeed adsorbed at the colloidal organic fraction of the sediment and one can note that this fraction is thorcughly mixed with the interstial water. According to the nature of the sediment, the interstitial water makes up a few % to 50 % of the "wet" sediment volume.

This means that, converting to this water fraction, we here can expect higher concentrations of these metals.

Although our study was limited to the influence of free metal ions and though it is very difficult to extrapolate to the complex sorption- and desorption reactions between sediment and interstitial water, with all possible chemical convertions from free to organically bound or complex metal ions, it is quasi certain that the various North Sea sediments, especially in the vicinity of dumping sites, and most Scheldt sediments, have considerably exceeded the toxicity threshold of Euplotes vannus.

Luckily this state of things is not yet reached as far as the organic pollutants (pesticides and PCB's) are concerned. The values found in water and sediment lie far below the inhibiting concentrations.

At the conclusion of this study, we have to mention, that the established toxicity thresholds for the separate pollutants only relate to those circumstances in which the pollutant was solely present.

The sea, however, is more and more a final destination site of often very complex dumpings of inorganic and organic pollutants which can therefore occur in enormously variable combinations of concentrations with respect to time and place.

It has already been proven often (a.o. by GRAY and VENTILLA on Cristigera) that the synergetic effect of various pollutants used together, can increase the toxicity a 10 to 100 fold, compared to the effect of the pollutant used solely.

We therefore intend to pay special attention to this problem in a subsequent study, and to test the influence of various pollutants in different combinations on Euplotes vannus which is a representative test organism of the benthic microfauna.

LITERATURE CITED

CARTER J.W. and CAMERON I.L. - Toxicity bioassay of heavy metals in water using Tetrahymena pyriformis. *Water Res., 1973, 7,(6), (in press).*

COOLEY N.R. - Effects of pesticides on Protozoa. *Paper read at the IV International Congress on Protozoology, Clermont-Ferrand, September 2-9, 1973.*

COOLEY N.R. and KELTNER Jr. J.M. - Effects of pesticides on estuarine ciliates. *Progr. Rep. Bur. Comm. Fish. Center Estuarine and Menhaden Res. Pesticide Field Station, Gulf Breeze, Fla. 1969, circular 335, 16-18.*

COOLEY N.R., KELTNER J.M. and FORESTER J. - Mirex and Aroclor ® 1254 : Effect on and Accumulation by Tetrahymena pyriformis Strain W. *J. Protozool., 1972, 19, (4), 636-638.*

COOLEY N.R., KELTNER Jr. J.M. and FORESTER J. - The Polychlorinated Biphenyls, Aroclors ® 1248 and 1260 : Effect on and Accumulation by Tetrahymena pyriformis. *J. Protozool., 1973, 20, (3), 443-445.*

DIETRICH G. and KALLE K. - General Oceanography, New-York, Interscience, 1963.

FENCHEL T. - The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. *Ophelia, 1967, 4, 121-137.*

GENERMONT T. - Quelques caractéristiques des populations de Paramecium aurelia adaptées au Chlorure de Calcium.
Protistologica, 1969, 5, 101-108.

GRAY J.S. and VENTILLA R.T. - Pollution effects on micro- and Meiofauna of sand.
Marine Pollution Bulletin, 1971, 2, (3), 39-43.

GREGORY W.W. Jr., REED J.K. and PRIESTER L.E. Jr. - Accumulation of parathion and DDT by some algae and protozoa.
J. Protozool., 1969, 16, 69-71.

MORGAN J.R. - Effects of Aroclor^{R} (a Polychlorinated Biphenyl) and DDT on cultures of an Alga, Protozoan, Daphnid, Ostracod and Guppy.
Bull. Environ. Contam. Toxicol., 1972, 8, (3), 129-137.

PERSOONE G. and DEPLAECIE M. - Influence de quelques facteurs écologiques sur la vitesse de reproduction du cilié hypotriche Euplotes vannus MULLER.
Protistologica, 1972, 8, (4), 427-433.

RUTHVEN J.A. and CAIRNS J. Jr. - Response of Fresh-Water Protozoan Artificial Communities to metals.
Journal of Protozoology, 1973, 20, (1), 127-135.

TINGLE L.E., PAVLAT W.A. and CAMERON I.L. - Sublethal cytotoxic effects of mercuric chloride on the ciliate Tetrahymena pyriformis.
J. Protozool., 1973, 20, 301-304.

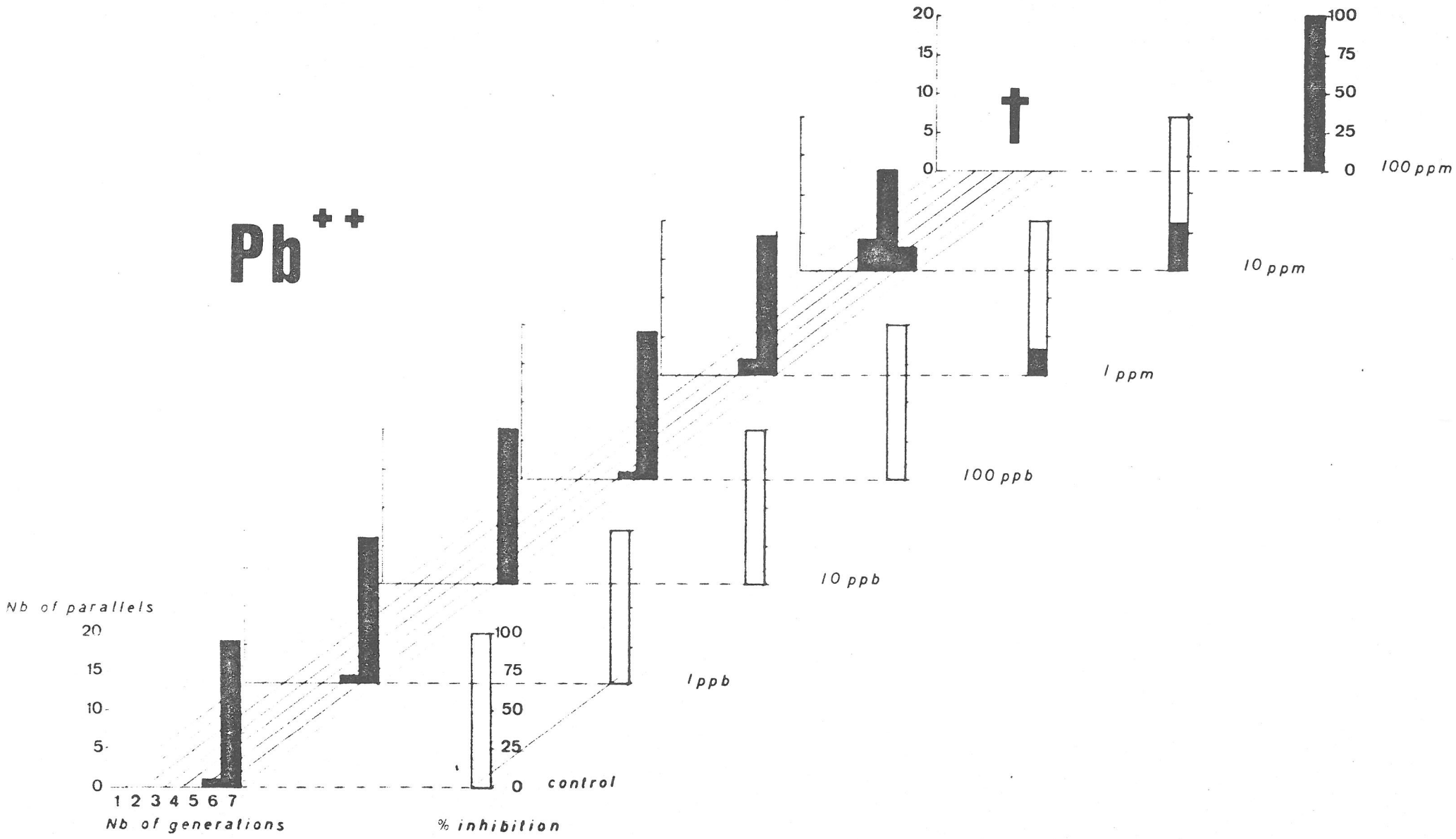
Pb++
Nb of parallels
20
15
10
5
0
1 2 3 4 5 6 7
Nb of generations
100
75
50
25
0
control
% inhibition
1 ppb
10 ppb
100 ppb
1 ppm
10 ppm
100 ppm

FIG. 1

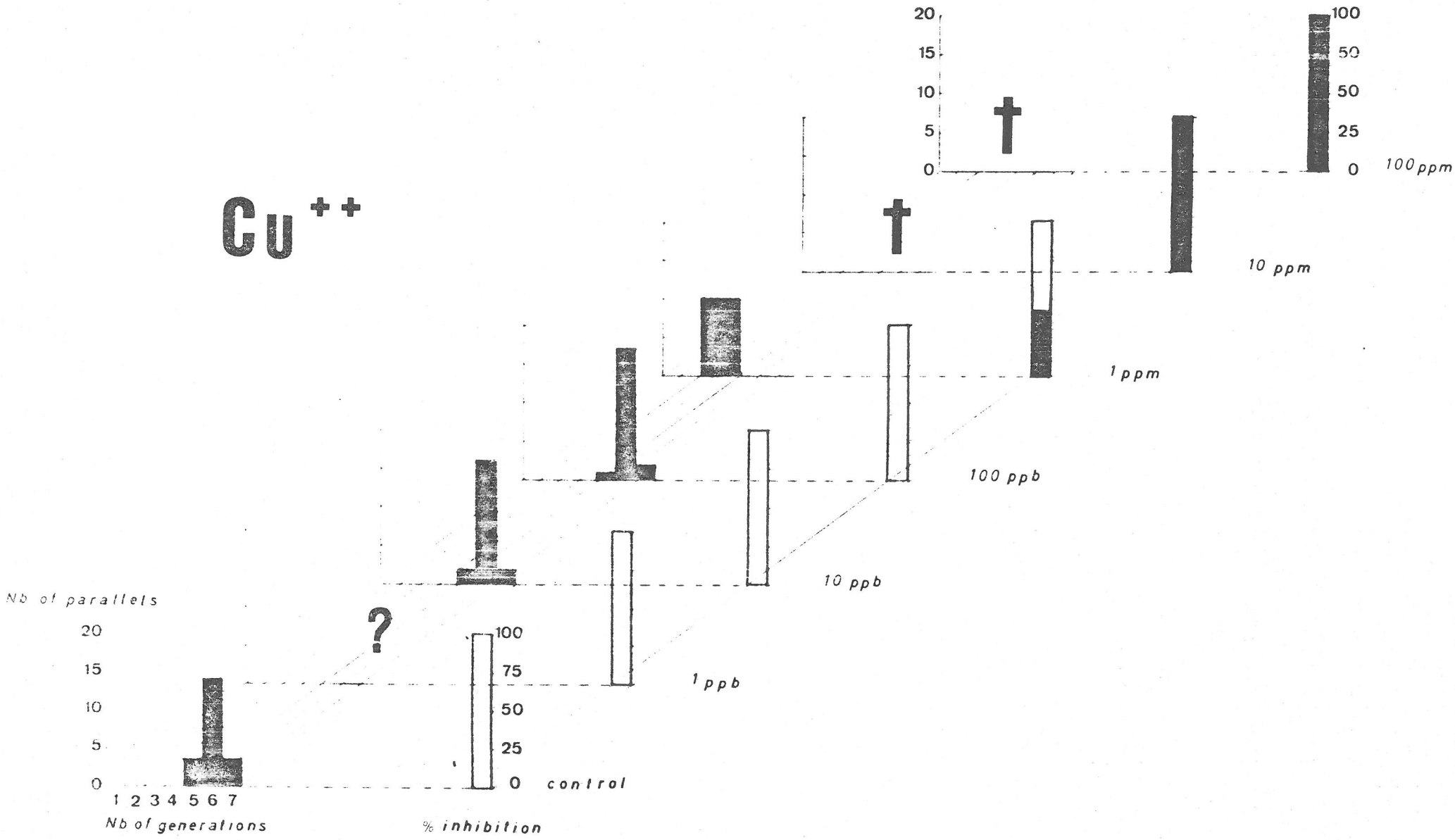
Cu ++
Nb of parallels
20
15
10
5
0
1 2 3 4 5 6 7
Nb of generations
?
100
75
50
25
0
control
% inhibition
1 ppb
10 ppb
100 ppb
1 ppm
10 ppm
100 ppm
†
†
20
15
10
5
0
100
50
50
25
0

FIG. 2

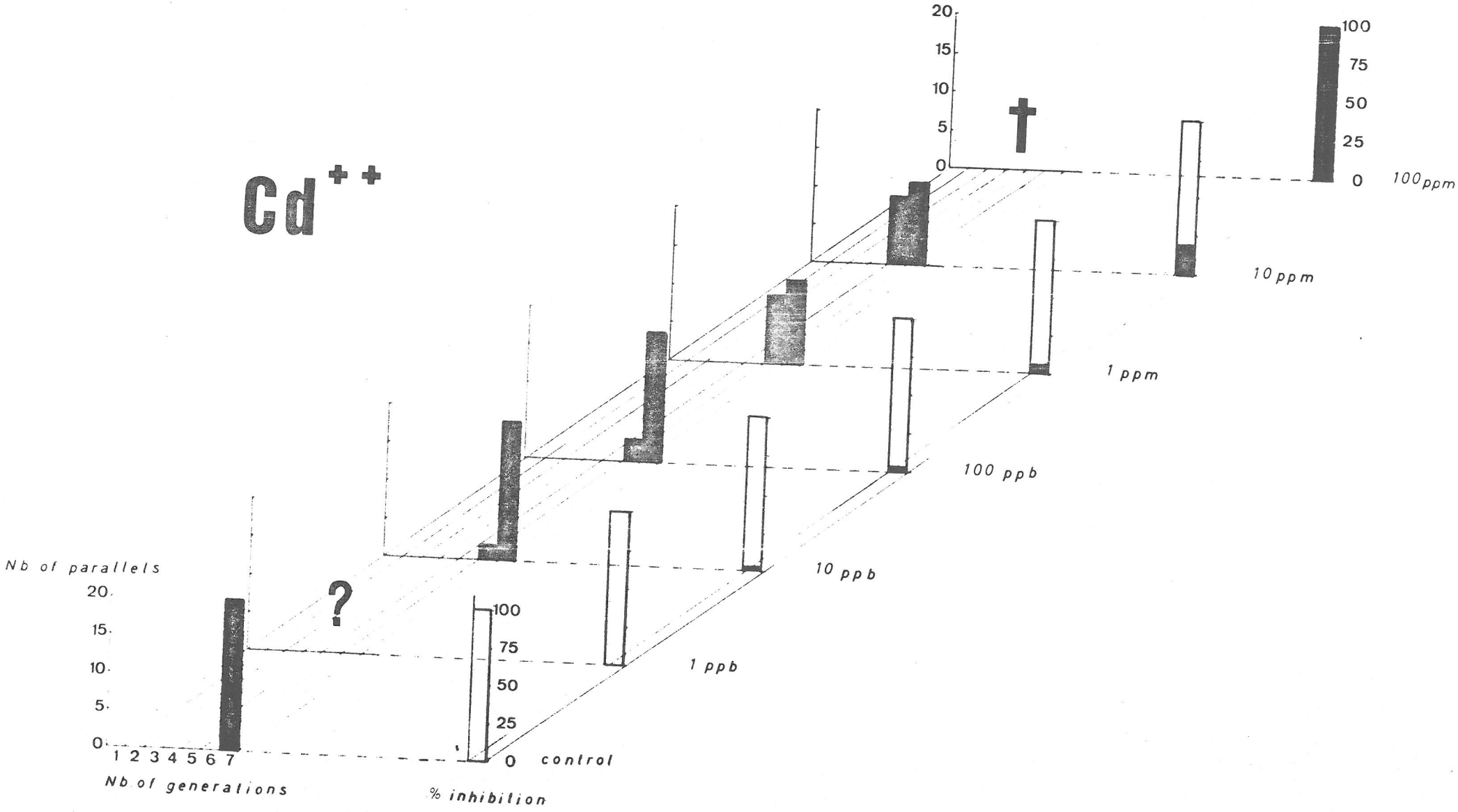
Cd++
Nb of parallels
20
15
10
5
0
1 2 3 4 5 6 7
Nb of generations
?
100
75
50
25
0
control
% inhibition
1 ppb
10 ppb
100 ppb
1 ppm
10 ppm
100 ppm
†

FIG. 3

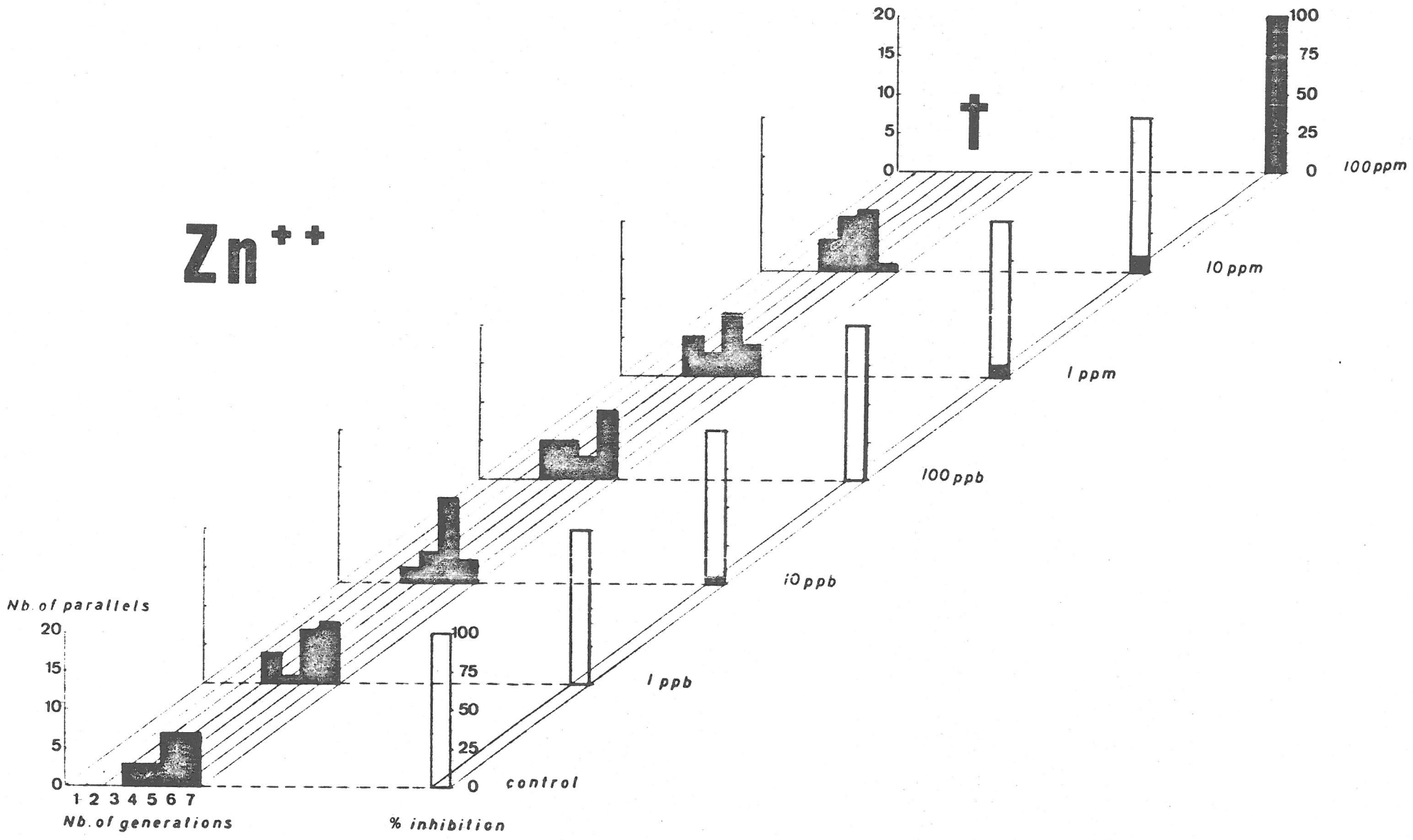
Zn++
Nb. of parallels
20
15
10
5
0
1 2 3 4 5 6 7
Nb. of generations
100
75
50
25
0
% inhibition
control
1 ppb
10 ppb
100 ppb
1 ppm
10 ppm
100ppm

FIG. 4

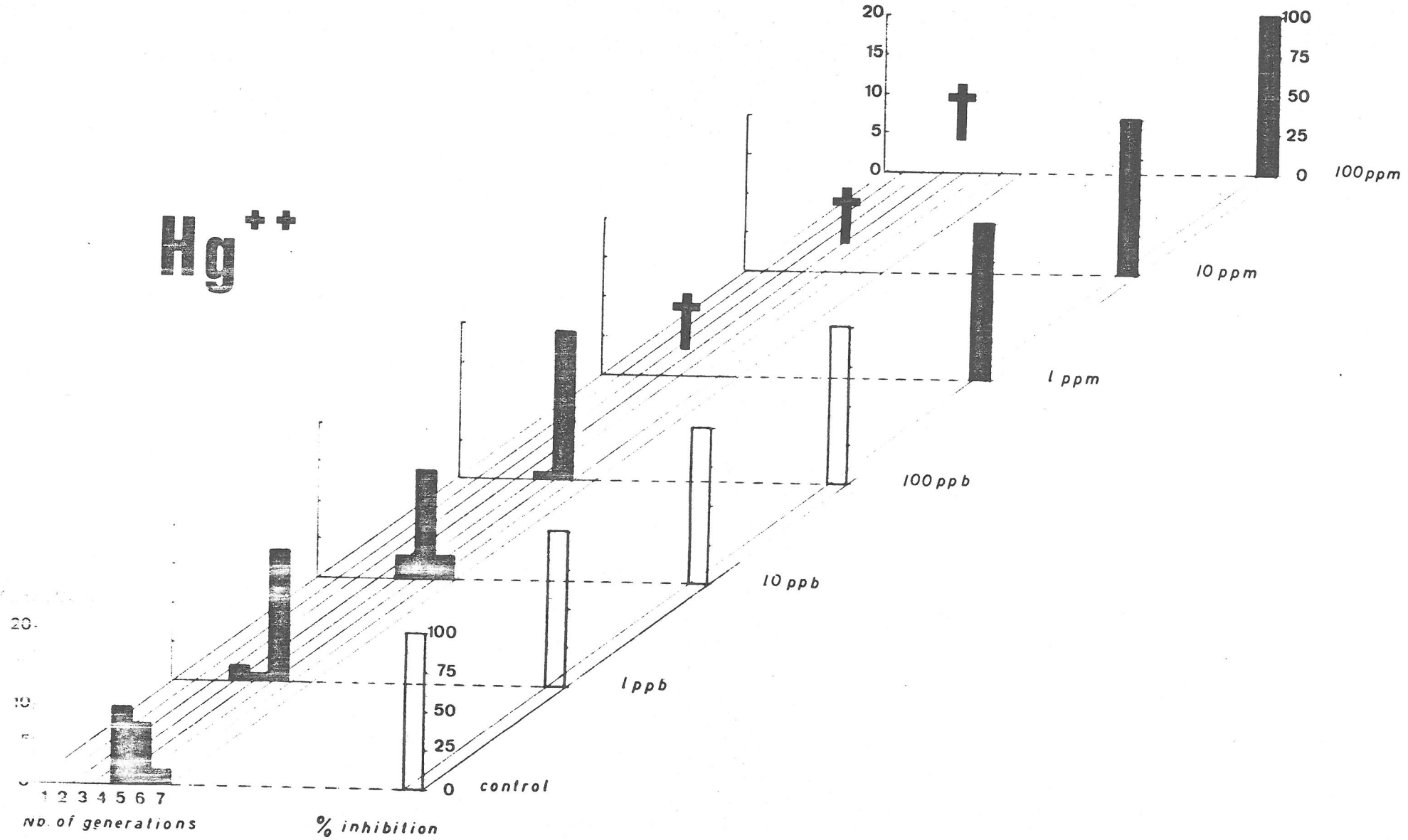
Hg++
20
15
10
5
0
100
75
50
25
0
100ppm
10 ppm
1 ppm
100 ppb
10 ppb
1 ppb
control
% inhibition
1 2 3 4 5 6 7
No. of generations

FIG. 5

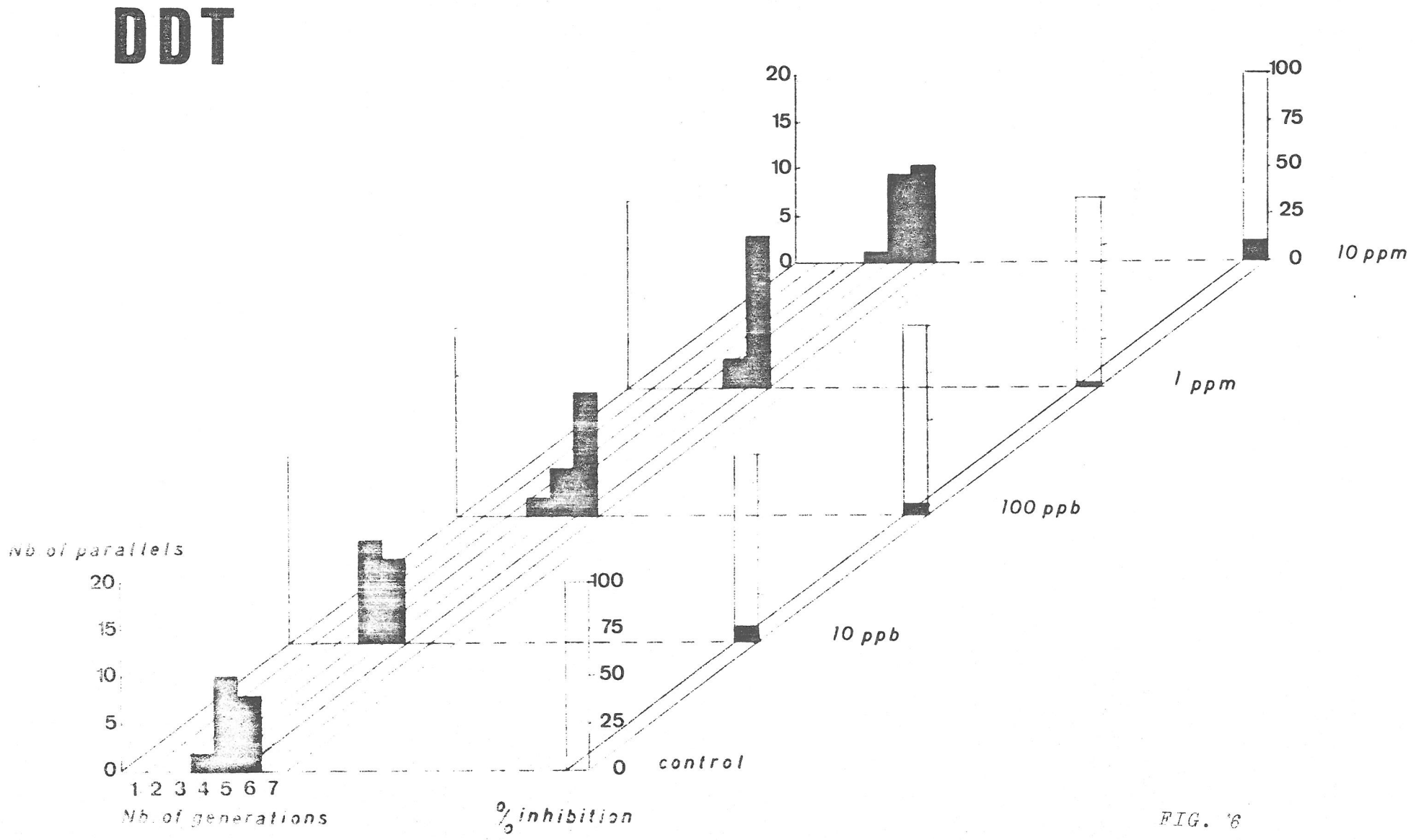
DDT
Nb of parallels
20
15
10
5
0
1 2 3 4 5 6 7
Nb of generations
100
75
50
25
0
control
% inhibition
10 ppb
100 ppb
1 ppm
10 ppm

FIG. 6

DDE

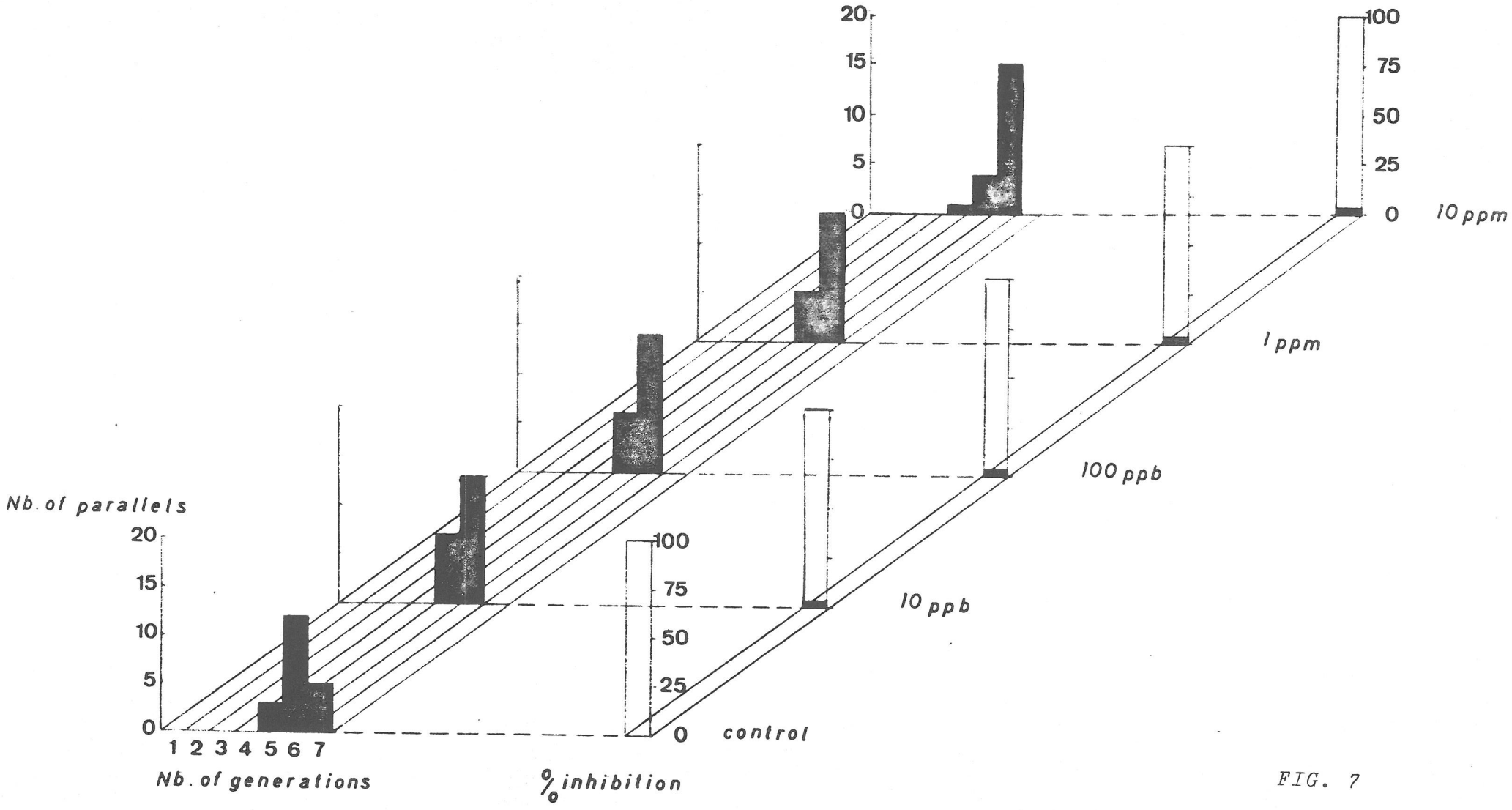
Nb. of parallels
20
15
10
5
0
1 2 3 4 5 6 7
Nb. of generations
100
75
50
25
0
control
% inhibition
10 ppb
100 ppb
1 ppm
10 ppm

FIG. 7

HEXACHLOR BENZENE

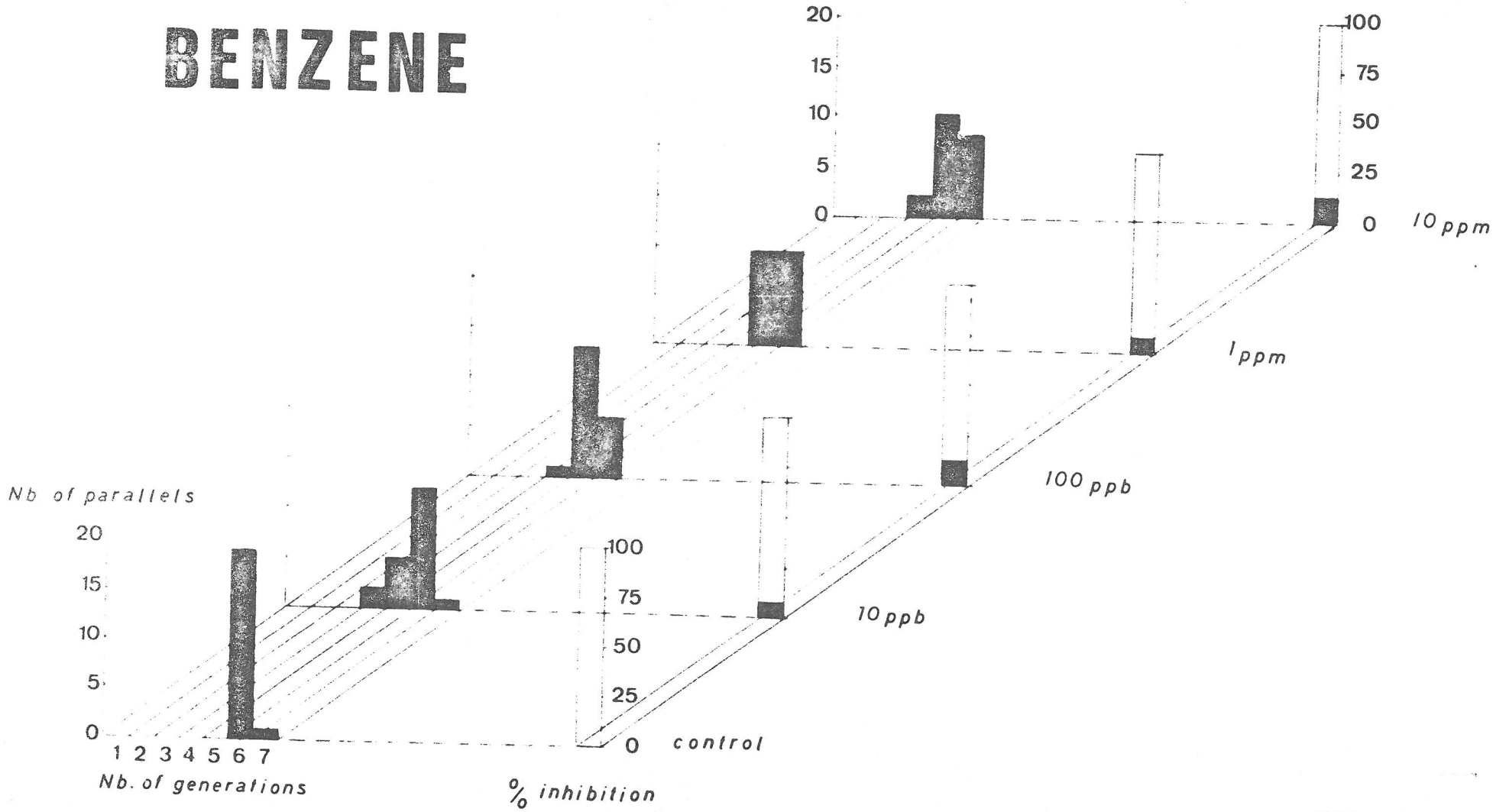

FIG. 8

HEPTACHLOR

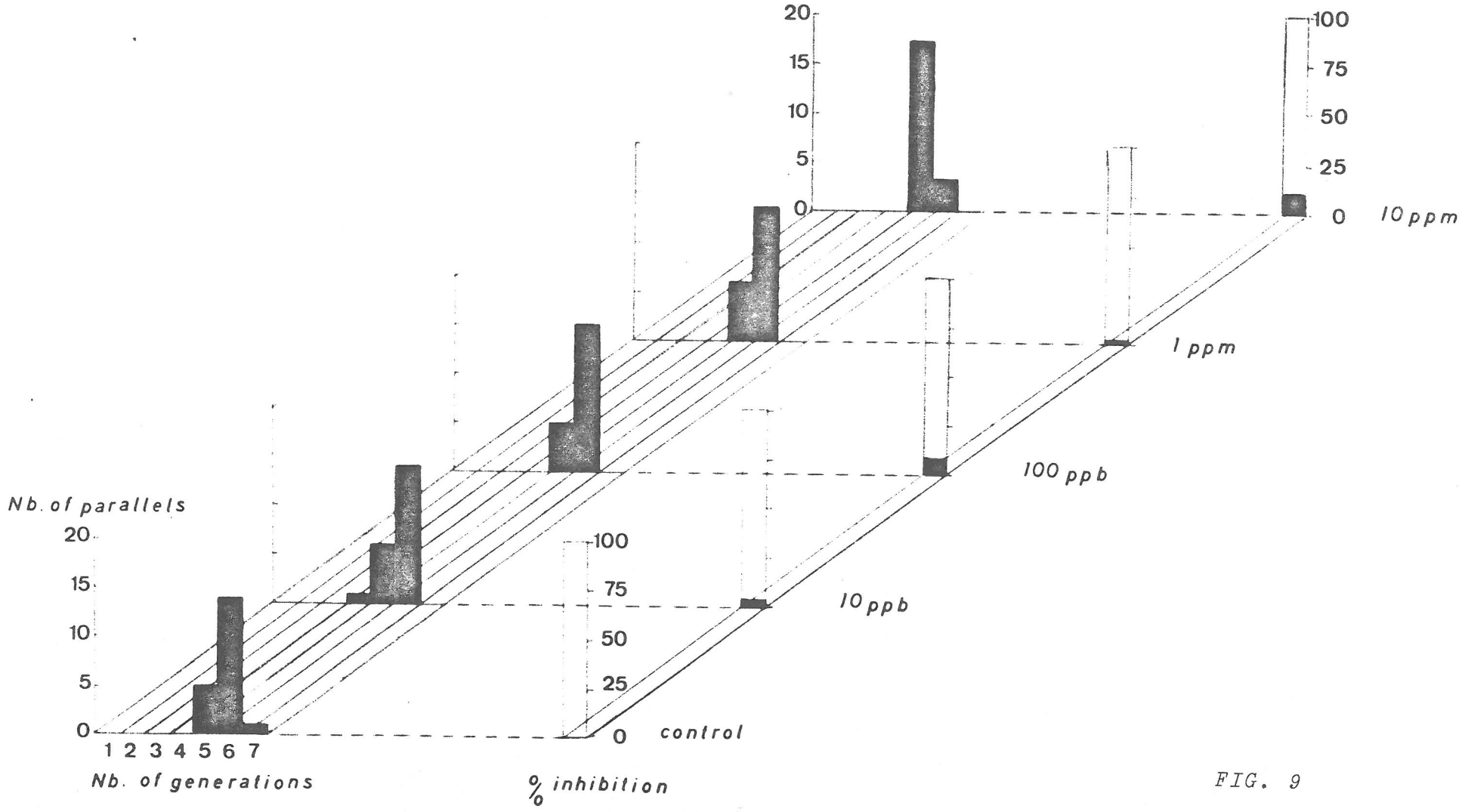

FIG. 9

HEXACHLOR CYCLOHEXANE

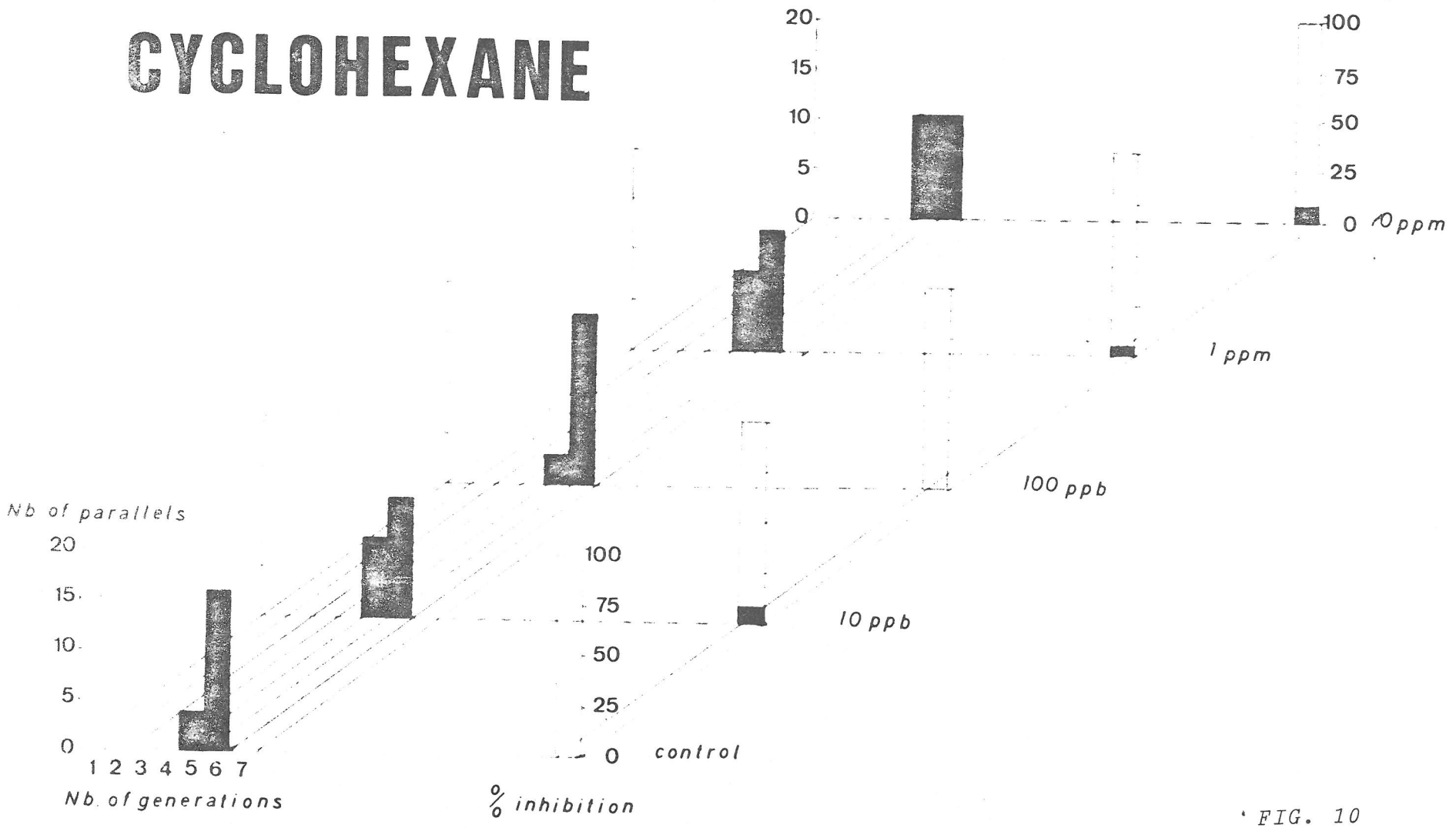

FIG. 10

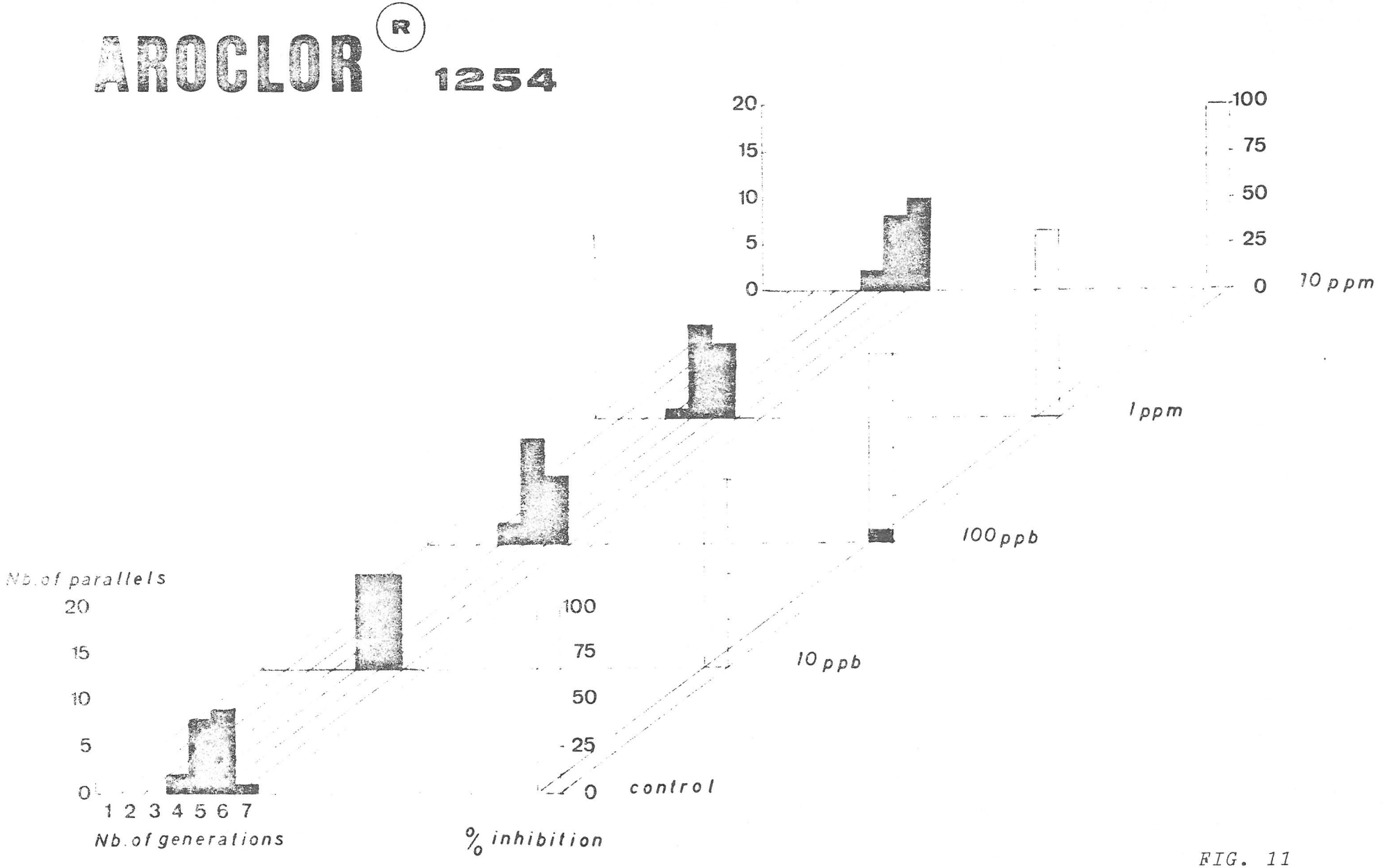
AROCLOR® 1254
Nb. of parallels
20
15
10
5
0
1 2 3 4 5 6 7
Nb. of generations
100
75
50
25
0
control
10 ppb
100 ppb
1 ppm
10 ppm
% inhibition

FIG. 11

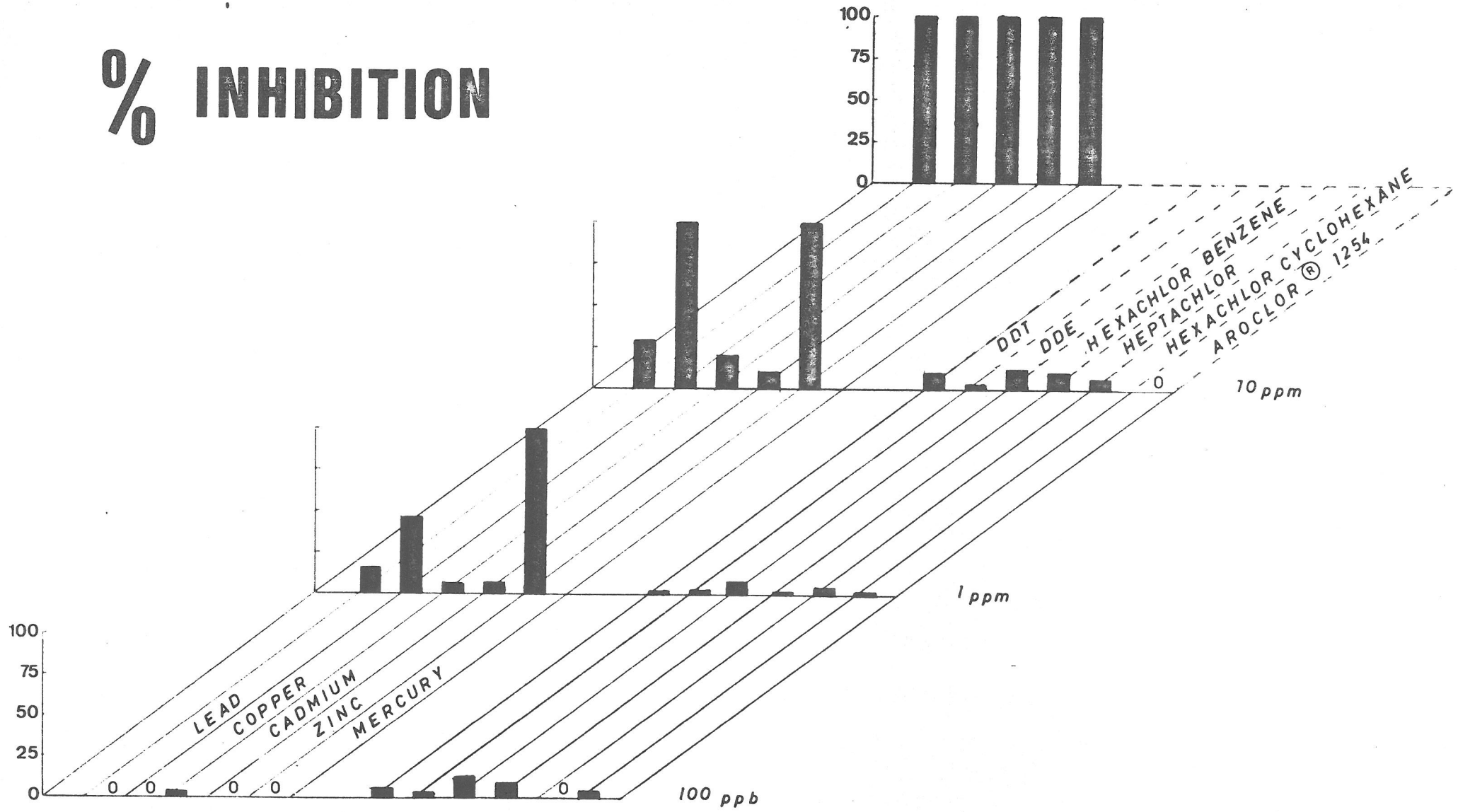
% INHIBITION
100
75
50
25
0
DDT
DDE
HEXACHLOR BENZENE
HEPTACHLOR
HEXACHLOR CYCLOHEXANE
AROCLOR ® 1254
0
10 ppm
1 ppm
100 ppb
LEAD
COPPER
CADMIUM
ZINC
MERCURY
0 0 0 0 0
HEAVY METALS
PESTICIDES + PCB

FIG. 12