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LABORATOIRE DE ZOOECOLOGIE DU SOL
INRA/CNRS, CEPE Louis Emberger
BP 5051 F-34033 MONTPELLIER France

LABORATOIRE DE CHIMIE ANALYTIQUE
USTL
Place Eugène Bataillon
F-34000 MONTPELLIER France

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CORRELATION OF LETHAL CONCENTRATIONS OF HEAVY METALS WITH TISSUE LEVELS OF EARTHWORMS

FINAL REPORT

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Scientific Senior
Professor J.Y. GAL
Dr Marcel B. BOUCHE

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P. BRUN (Full time) 2nd year PHD
ABDUL RIDA A.M. (Half time) 2nd year PHD

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I. INTRODUCTION

The objectives of this reasearch are:

1) Objectives

a. ~~To~~ establish the LC50 of the heavy metals ~~Cd, Cu, As and Hg~~ Cadmium, Copper, Arsenic and Mercury as well as a Cu/Cd mixture in a ratio equal to LC50 Cd/LC50 Cu, employing a chemically defined medium such as "Artisol";

b. ~~To~~ improve procedures for heavy metal bioavailability studies in the field;

c. ~~To~~ improve/develop test procedures using a chemically defined medium and "food" in 28-day uptake studies;

d. ~~To~~ analyze the tissues of the surviving test earthworms as well as those from media of lesser concentrations, blanks and background worm stock;

e. ~~To~~ relate the LC50 concentrations of contaminants with the tissue contaminant levels in the earthworms utilized in the toxicity test; and

f. ~~To~~ develop first generation interpretations relating soil contaminant levels and bioavailability, and to interpret the fate and transport of heavy metal contaminants. (All)

2) Difficulties

Between the launching of the program and the first money compensation for services, the decrease exchange rate of French Francs vs Dollar was 1 $ = 10 FF to 1 $ = 5.8 FF. This lost for costs and service of more 40 % curtailed our possibilities to reach all initial objectives (difficulties in As and Hg analysis facilities and postponement in bioavailability studies in the field).

3) Work steps

The report deals with :

a) A description of material and methods used for our work, including animals, toxicity test procedure and analytical technics employed for sample mineralization and measurements.

b) A study on heavy metal toxicity mentioned in the contract i.e. : mercury, arsenic, cadmium and copper. We will find out the 50 % lethal concentrations (LC50) and the smallest concentrations having an effect on earthworms or 5 % lethal concentrations (LC5). Thus, we will be able to class these chemicals in increasing toxicity.

c) A discussion on earthworm tissues analytical results. The analysis of the tissues of the surviving test earthworms as well as those from media of lesser concentrations, blanks, and background worm stock has been limited to copper and cadmium. In fact, mercury and arsenic request special analytical device that we could not buy for the financial reason given above.

II. MATERIAL AND METHODS

II.1. Background worm stock

We took a well homogenized earthworm culture to get the most reproductible results as possible.

Besides, the chosen species must present neither sexual rest, nor diapause.

Thus, we have used Eisenia fetida P1756, an epigeous earthworm. More precisely we used E. fetida subspecies andrei because the isoenzyme studies of genetical diversity (ROBOTTI, 1983) demonstrate the low variability of this taxon. It is a small species with high reproduction ratio easily cultured in organic substrates (rubish, compost, litter). We breeded it in a composted sheep dung.

Characteristics :

Length : 50 to 120 mm
Width : 2 to 4 mm
Weight : 200 to 400 mg
60 to 120 segments
Pigmentation : red-violet.

Thermal optimum species is about 22-23°C.

II.2. Toxicity test

Method used for the test is based on the European Standard for ecotoxicity studies in earthworms (EEC Directive 19/831).

The test is carried out by two different steps. The first one is the preliminary test, to find out the contaminant concentration range giving between 0 and 100 per cent of mortality among earthworms, and the second one regroups the different other tests which are necessary to get intermediary mortality percentages between 0 and 100 %.

II.2.1. Materials and organisms

We put adult earthworms in an artificial media called "Artisol", mixed with different concentrations of the substance to test.

The surviving earthworms are counted 14 days later.

a) Materials

The materials include test containers and test substrate. Test containers used are plastic boxes about 3 liters (21.5 cm x 15 cm x 10.5 cm) with perforated covers to allow aeration.

Test substrate is the medium inside the box. We call it "Artisol" and it is composed of two elements :

- a skeleton of glass balls (of about 2 cm in diameter) : 1425 g of glass balls (+/- one glass ball) per container (about 100 balls per box),
- a matrix which is composed of 90 g of a peculiar silica per test container (trade mark : "levilite") deshydrated in an oven at 105°C and rehydrated with 215 ml of deionized water containing required quantity of contaminant to test disolved in it.

b) Organisms

Test organisms used in toxicity tests must normally be chosen among Eisenia fetida adults, i.e. at least two months old, with clitellum and weight between 300-600 mg. They must have approximately the same size and weight. Before test, they are washed with tap water, rinsed with dionized water and placed in "artisol" without contaminant at the test temperature (about 20° C) during 48 hours to get them cleared.

II.2.2. Test procedure

Each heavy metal or contaminant must be prepared at different concentrations. It is also necessary to prepare for each test a reference box or "blank", in the same conditions as the other boxes but without toxic components.

Contaminants to study are heavy metals : cadmium, copper and mercury, and arsenic. We chose following chemicals : cadmium chloride 2.5 hydrate ($CdCl_2$, 5/2 H_2O), copper chloride dihydrate ($CuCl_2$, $2H_2O$), mercury chloride ($HgCL_2$) and sodium meta-arsenite ($NaAsO_2$). Molecular weights of these chemicals and their components with weight ratio are given in **Table 1**.

TOXIC COMPONENTS	CHEMICALS USED	TOXIC COMP. WEIGHTS IN G	CHEMICAL MOL. WEIGHTS IN G	% OF TOXIC COMP. IN CHEMICALS
Cu	CuC12,2H20	63.546 G	170.48 G	37.27 %
As	NaAs02	74.922 G	129.91 G	57.67 %
Hg	HgC12	200.59 G	271.50 G	73.88 %
Cd	CdC12,2.5H20	112.40 G	228.34 G	49.22 %

Table 1 : MOLECULAR AND ATOMIC WEIGHTS OF CHEMICALS USED AND TOXIC COMPONENTS WITH THEIR PERCENTAGE IN CHEMICALS

These chemicals are all water-soluble and for this reason they are firstly disolved in deionized water to get the proper concentration, calculated in mg of metal or contaminant (Cd, Cu, Hg or As) per mg of dry weight silica.

In each test box, we pour 215 ml of water containing contaminant. Then we add 90 g of silica powder deshydrated at 105° C and we mix all together to make a homogenous matrix with a good contaminant repartition. Finally we incorporate the glass balls with the matrix and knead the whole. Boxes are ready to host earthworms, previously cleared in "Blank" Artisol during 2 days as described before. We place 10 earthworms in each container onto the medium surface and put boxes in a chamber at 20°C +/- 2°C in continous dark, and air humidity 70-90 % RH.

II.3. Sample analysis

Samples are earthworms and for a few of them "Artisol".

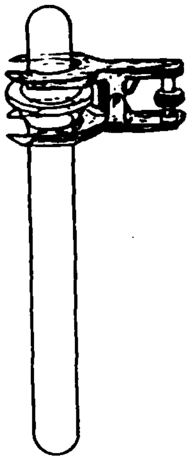
We used the same mineralization method for all samples and the same analytical technics.

Our laboratory is equiped with a flame atomic absorption spectrometer, thus we chose this method for cadmium and copper analysis. However, we could not analyse mercury and arsenic which require a special equipment (Hydride source) that we do not have.

We tried for arsenic to make a coloured-complex with silver diethyldithiocarbamate in pyridin and to measure it absorbance with a colorimeter but we did not get good results.

Besides it is necessary to use special hermetic tubes for mercury mineralization, because this metal is very volatile (see **fig. 1**).

Fig 1 : MINERALIZATION TUBE FOR MERCURY ANALYSIS



III.3.1. Mineralization

Before mineralization, samples are weighted and dried in glass flask of 30 or 65 ml in oven at 105°C during 24 h. Then, they are cooled in desiccator during 1 hour and weighted again, to know sample percentages in water. Mineralization and drying are done in the same flasks. We only add nitric acid (Merck, for analysis) at 65 % (copper and cadmium < 0,000001 %) in a rate of 5 ml HNO_3 for 100 mg dry weight. Flasks are then closed with plastic caps not susceptible to acid vapours and leaved at 70°C during 24 hours. After mineralization, samples are diluted with deionized water in proper flasks to get an acid rate of 10 % HNO_3 at 65 % (25, 50 or 100 ml according to HNO_3 quantity used for mineralization, respectively 2.5, 5 or 10 ml HNO_3 at 65 %).

Finally, they are stored in polyethylene flasks before analysing.

II.3.2. Analysis

From initial cadmium and copper solutions at 1 g metal/l (Standard solutions Merck), which we can keep for a long time (6 months to one year) in a cool place, we make less concentrated solutions. They must also contain 10 % nitric acid at 65 % to be similar to samples. The standards are prepared between 0 and 2 mg/l in cadmium or copper (see **Table 2**).

STANDARD CONCENTRATION IN PPM Cd OR Cu	VOLUME IN µL OF INITIAL SOLUTION 1 G/L IN METAL	VOLUME OF NITIC ACID AT 65% (IN ML)	DEIONIZED WATER (IN ML) COMPLETED TO
0 PPM	0 µL	10 ML	100 ML
0.5 PPM	50 µL	10 ML	100 ML
1 PPM	100 µL	10 ML	100 ML
1.5 PPM	150 µL	10 ML	100 ML
2 PPM	200 µL	10 ML	100 ML

Table 2 : COMPOSITION OF Cd AND Cu STANDARD SOLUTIONS

With these standard solutions, we can establish the relation between absorbance and concentration, and draw the curves giving absorbance for different metal concentrations and for a determined adjustment at the atomic absorption spectropnotometer (see **Table 3** below).

PARAMETERS	CADMIUM	COPPER
INSTRUMENT MODE	ABSORBANCE	ABSORBANCE
CALIBRATION MODE	CONCENTRATION	CONCENTRATION
MEASUREMENT MODE	INTEGRATION	INTEGRATION
LAMP POSITION	4	3
LAMP CURRENT (mA)	3	4
SLIT WIDTH (nm)	0.5	0.5
SLIT HEIGHT	NORMAL	NORMAL
WAVELENGTH (nm)	228.8	324.8
FLAME	AIR-ACETYLENE	AIR-ACETYLENE
SAMPLE INTRODUCTION	AUTO NORMAL	AUTO NORMAL
DELAY TIME	5	5
TIME CONSTANT	0.05	0.05
MEASUREMENT TIME (sec)	5.0	5.0
REPLICATES	2	2
BACKGROUND CORRECTION	OFF	OFF
AIR FLOW (L/min)	13.5	13.5
ACETYLENE FLOW (L/min)	1.5	1.5
RINSE RATE	1	1
RINSE TIME (sec)	5.0	5.0
RECALIBRATION RATE	0	0
RESLOPE RATE	0	0

Table 3 : SETTING UP OF ATOMIC ABSORPTION SPECTROMETER

For this reason, these curves must be drawn for each new use of the spectrometer. The curves are shown for copper and cadmium in **figures 2 and 3**. Thus, we can find out sample concentration in one of these metals, corresponding on curve, to its absorbance reading.

II.4. Improvements for heavy metal bioavailability studies in the field

The objective was :
- to precise field practices of sampling,
- to compare concentrations of heavy metals in soil to the various earthworm species available in the sampling site.

To optimize interpretation, we adopt "points procedure" i.e. we sampled each very local geographical point for soil and earthworms living closely associated (in practice few square decimeters of soil were hand sorted for earthworms and the same soil was sampled).

The objective is to create a matrix of 1 to n points X 1 to n variables with a maximum of points covering a great range of values for each variables.

The variables are local soil properties (texture variables, pH, C, N, type of soil, ...), local type of vegetation, individual analysis of each earthworm for heavy metals, soil hevay metal analysis.

The points were chosen to sample various soil types (more exactly an acid group on schist-granit rock bed and a neutral to basic group on calcareous rocks) and typical pollutions : one "unpolluted" series, one urban series, one industrial pollution (both through water and fume losses) and one on a mining spoil site (acid soils with lead and cadmium pollution).

More than two hundred points has been sampled, one thousand earthworms and some soil characteristics analysis has been made.

Because we use for both soil and individual earthworm the same sample, the same mineralization and the same heavy metal mineralization solution to estimate the various pollutants, we chose to delay this type of analysis because we were unable to estimate mercury which require special analytical devices (see above item 2 of 1).

III. TOXICITY STUDY

III.1. Toxicity definition

We can define a toxic contaminant as any biological, chemical or physical factor creating a potential pollution source.

Generally they penetrate organism by respiration teguments or alimentation.

With earthworms, we only have toxicity by contact (cutaneous passage) or by assimilation (digestive tract).

Earthworm respiration is cutaneous. Thus there is no contamination possible by a specific inhalation way.

The toxic effects of substrate contaminants appear in physiological troubles which ultimate phase is death.

Fig 2 : CADMIUM STANDARD CURVE

SAMPLE	CONCENTRATION PPM	% RSD	MEAN ABSORBANCE	READINGS	
BLANK	0.000		0.002	0.002	0.002
STANDARD 1	0.500	0.4	0.207	0.207	0.208
STANDARD 2	1.000	0.4	0.380	0.379	0.381
STANDARD 3	1.500	0.4	0.530	0.528	0.531
STANDARD 4	2.000	0.4	0.660	0.658	0.662

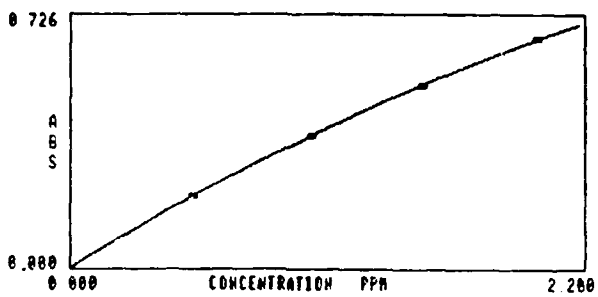
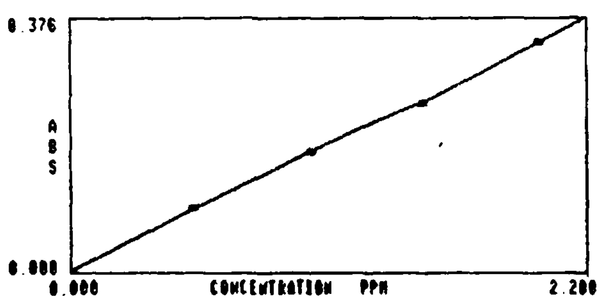


Fig 3 : COPPER STANDARD CURVE

SAMPLE	CONCENTRATION PPM	% RSD	MEAN ABSORBANCE	READINGS	
BLANK	0.000		0.001	0.001	0.000
STANDARD 1	0.500	0.2	0.093	0.093	0.093
STANDARD 2	1.000	0.3	0.180	0.180	0.180
STANDARD 3	1.500	0.1	0.253	0.253	0.254
STANDARD 4	2.000	0.3	0.343	0.344	0.343



We can distinguish two different sorts of toxicity :
- long term toxicity,
- acute toxicity.

Long term toxicity can involve malformations, changes in behaviour, reproductive function modifications whereas acute toxicity involves quickly serious physiological troubles and contaminated animals death (swelling appearance on earthworms body, fragility, bleeding, self-cutting of animals). Acute toxicity measure is considered as a reference for risks evaluation including long term toxicity.

III.2. Toxicity measure

It is made with toxicological tests which enable to quantify sensibility or resistance degree of animals towards toxic substances.

We will characterize heavy metals toxicity towards earthworms with 2 criteria :

- LC50 (50 lethal concentration) : toxic contaminant concentration in medium killing 50 % of animals,
- LC5 : contaminant concentration involving 5 % of mortality also called the smallest concentration having an effect on earthwomrs.

All concentrations will be expressed proportionate to pollutants and substrate dry weights.

For the determination of these 2 concentrations, we find out animals mortality for differnt contaminant concentrations in medium. Classical mathematical representation of this phenomenon in toxicology is a sigmoïd function from which it is difficult to get LC50 and LC5. However this function can be transformed into a linear function by the log-probit model.

LOWY et al. (1980) give and discuss the main characteristics of this model. At each contamiant concentration we associate probability to get no effect with this concentration (surviving) transformed in probit unit (see **Annex I**).

Normally six values are necessary to get the regression line : 4 values for intermediary mortalities and 2 values for extreme mortalities (null and total).

If we observe dead animals in the blank and take into account only mortality involved by contaminant we calculate the corrected mortality.

$$P'_d = \frac{P_d - Po}{1 - P_O}$$

P'_d = corrected mortality
Po = observed mortality in blank
P_d = observed mortality after treatment

The blank mortality must not exceed 10 % to give a valid correction.

Then corrected mortability is changed into corrected surviving (100

% minus % mortality) and probit units according to the table given in **Annex 1.**

Once concentrations values are obtained in "log" and surviving percentages in "Probit" we calculate the regression line equation and correlation coefficient. A test of this coefficient enables to know the significant level

$$F = r\sqrt{\frac{n - 2}{1 - r^2}}$$

r : correlation coefficient
n : data number

Calculated F coefficient value is compared with theoritical F value in Fischer-Snedecor table (in **Annex 2**). We get then the significant level. We will accept the results if it is lower than 5 %.

thus, we have a mathematical model : log-probit model to find out LC50 and LC5 and to class the test pollutants according to these 2 factors.

III.3. Toxicity of studied contaminants
(Cd, Cu, Hg and As)

To determine intermediary lethal range for the studies contaminants, we must firstly carry out a preliminary test i.e. a first test series with concentrations for instance of 10, 100, 1000 and 10000 ppm.

A second test series in a more restricted range according to the results of the first one will give us intermediary values (we choose the highest giving 0 % mortality and the lowest giving 100 % mortality to carry out the second test). Generally, we must prepare so many series as necessary to get enough intermediary values (3 to 4 values between 10 and 90 % mortality).

Besides each concentration must normally be repeated 4 times according to the standard but we have only prepared it once lacking of materials (Balls and plastic boxes). The fact to use repeatidly the same standardized medium, with a restriction of concentrations and to use precise mathematical fittings increase in another point of view the confidence on results.

III.3.1. Preliminary tests

Thus, we carried out preliminary tests. The percentages of mortality are presented in following table (**Table 4**) for the different contaminants and their concentrations in "Artisol" medium (10, 100, 1000 and 10000 ppm).

CONCENTRATIONS CONTAMINANTS	10 PPM	100 PPM	1000 PPM	10000 PPM
Cu	0 %	70 %	100 %	–
As	0 %	0 %	100 %	–
Hg	0 %	20 %	100 %	–
Cd	–	0 %	90 %	100 %

Table 4 : RESULTS (% of earthworms mortality) OF PRELIMINARY TESTS OF 06-16-87 (2 WEEKS)

III.3.2. Complementary tests

a) Mercury toxicity studies

Two tests has been carried out for mercury : 07-08-87 test (2 weeks length) and 09-02-87 test (4 weeks length). Results are shown below **(tables 5 and 7)**.

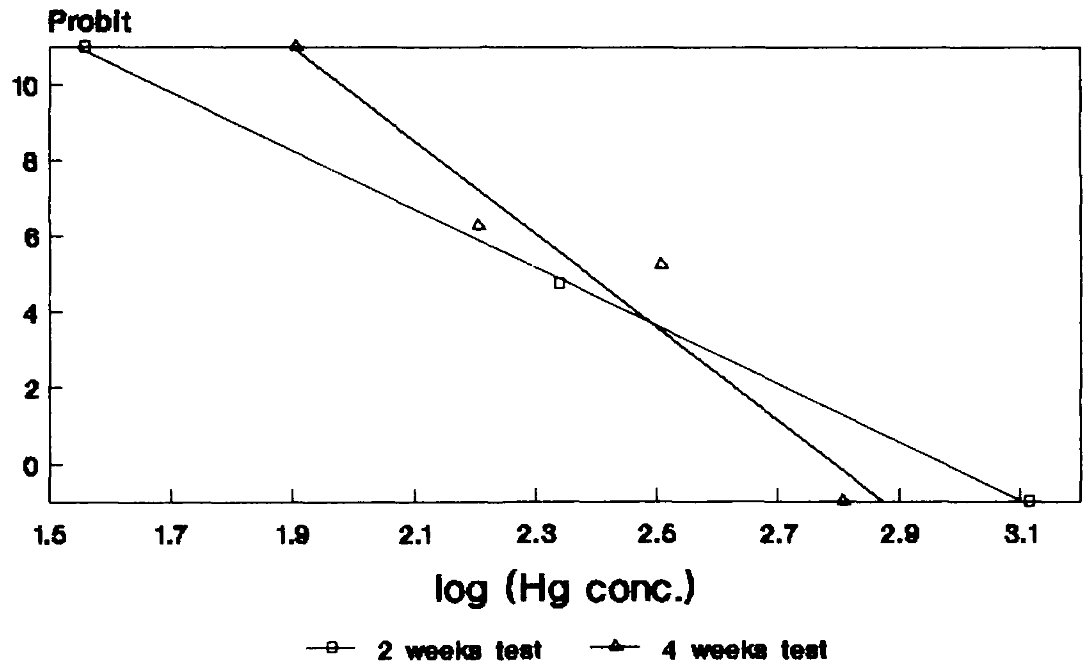
* 07-08-87 test (2 weeks length)

ARTISOL CONC. IN PPM OF DRY WEIGHT	log OF ARTISOL CONC. IN PPM OF DRY WEIGHT	SURVIVING (N) WORMS NUMBER AFTER 14 DAYS	PERCENTAGE P OF MORTALITY P=(10-N)*10%	PROBIT VALUE
0 PPM	–	10	0 %	11
1 PPM	0 PPM	10	0 %	11
6 PPM	0.778 PPM	10	0 %	11
36 PPM	1.556 PPM	10	0 %	11
216 PPM	2.334 PPM	4	60 %	4.746
1296 PPM	3.113 PPM	0	100 %	-1

Table 5 : RESULTS OF MERCURY TEST OF 07-08-87 (2 WEEKS LENGTH)

We draw these results on a graph : Probit values function of the medium concentration logarithm (see **figure 4**). Then we find out the regression line between all points (see following **table 6**)

Fig 4 : Earthworms toxicity tests
in artisol contaminated with mercury



date of 2 weeks test : 07-08-87
date of 4 weeks test : 09-02-87

REGRESSION LINE EQUATION	Y = -7.707 X + 22.906 (with 36,216,1296 ppm)
CORRELATION COEFFICIENT (r)	r = -0.999
CORRELATION (n=3) COEFFICIENT TEST	$t = (\lvert r \rvert \sqrt{n-2})/(\sqrt{1-r^2})$ t = 40.30 (d.of.f=n-2=1)
LC 50 IN PPM OF DRY WEIGHT ARTISOL	210.546 PPM ≈ 211 PPM
LC 5 IN PPM OF DRY WEIGHT ARTISOL	128.801 PPM ≈ 129 PPM

Table 6 : RESULTS ANALYSIS OF MERCURY TEST OF 07-08-87 (2 WEEKS LENGTH)

Linear regression gives a high correlation coefficient r = 1. However, it has been calculated with only 3 points whereas 6 would have been necessary. Besides, correlation coefficient test unables to deduct from t distribution table for one degree of freedom (see Annex 3) that this test is significant with a probability p < 0,025, i.e. with an error risk a < 2.5 %.

* 09-02-87 test (4 weeks length)

ARTISOL CONC. IN PPM OF DRY WEIGHT	log OF ARTISOL CONC. IN PPM OF DRY WEIGHT	SURVIVING (N) WORMS NUMBER AFTER 28 DAYS	PERCENTAGE P OF MORTALITY P=(10-N)*10%	PROBIT VALUE
0 PPM	–	10	0 %	11
40 PPM	1.602 PPM	10	0 %	11
80 PPM	1.903 PPM	10	0 %	11
160 PPM	2.204 PPM	9	10 %	6.282
320 PPM	2.505 PPM	6	40 %	5.253
640 PPM	2.806 PPM	0	100 %	-1
1280 PPM	3.107 PPM	0	100 %	-1

Table 7 : RESULTS OF MERCURY TEST OF 09-02-87 (4 WEEKS LENGTH)

These results are drawn on a graph (see **fig.4**) and the linear line is calculated (see below **table 8**).

REGRESSION LINE EQUATION	Y = -12.302 X + 34.349 (with 80,160,320,640 ppm)
CORRELATION COEFFICIENT (r)	r = -0.968
CORRELATION (n=4) COEFFICIENT TEST	$t = (\|r\|\sqrt{n-2})/(\sqrt{1-r^2})$ t = 5.483 (d.of.f=n-2=2)
LC 50 IN PPM OF DRY WEIGHT ARTISOL	243.05 PPM ≈ 243 PPM
LC 5 IN PPM OF DRY WEIGHT ARTISOL	178.64 PPM ≈ 179 PPM

Table 8 : RESULTS ANALYSIS OF MERCURY TEST OF 09-02-87 (4 WEEKS LENGTH)

Correlation coefficient shows a very high correlation between mortality and mercury concentration in "Artisol" medium because $r > 0.95$. Moreover, t test indicates, for two degrees of freedom that correlation is significant with a probability $p < 0.05$ because $t > 4.303$ (error risk $a < 5\%$).

Obviously from these 2 mercury toxicity tests (lengths : 14 and 28 days), we can notice that mercury LC50 is about 230 ppm and that LC5 values (about 150 ppm) are not very far from LC50 values because of high regression line slopes.

It would have been interesting to do again test, to get more points for the regression line (at least 4 intermediary points). It would have been necessary to proceed again between 100 and 600 ppm (for instance : 100, 200, 300, 400, 500 and 600 ppm). However, we have not time enough to do it.

b) Arsenic toxicity study

A first complementary test (07-08-87, 2 weeks length) has been necessary for Arsenic to reduce the range to study to 100 to 200 ppm (see following table 9)

* 07-08-87 test (test length : 2 weeks)

ARTISOL CONC. IN PPM OF DRY WEIGHT	SURVIVING (N) WORMS NUMBER AFTER 14 DAYS	PERCENTAGE P OF MORTALITY P=(10-N)*10%
0 PPM	10	0 %
100 PPM	10	0 %
200 PPM	0	100 %
400 PPM	0	100 %
800 PPM	0	100 %
1600 PPM	0	100 %

Table 9 : RESULTS OF ARSENIC TEST OF 07-08-87 (2 WEEKS LENGTH)

After this test, we carried out 4 other toxicity tests of 1 to 4 respective lengths and for concentrations in arsenic between 100 and 200 ppm. Test results are presented in the following table 10.

* 05-10-88 tests (test length : 1, 2, 3 and 4 weeks)

ARTISOL CONCENTRATION IN PPM OF DRY WEIGHT	0 PPM	100 PPM	120 PPM	140 PPM	170 PPM	200 PPM
log(CONC.) IN PPM	–	2	2.079	2.146	2.230	2.301
SURV. WORMS NUMBER (1W)	10	4	3	1	0	0
PERCENTAGE OF MORTALITY	0 %	60 %	70 %	90 %	100 %	100 %
PROBIT VALUE	11	4.746	4.476	3.178	-1	-1
SURV. WORMS NUMBER (2W)	10	8	0	0	0	0
PERCENTAGE OF MORTALITY	0 %	20 %	100 %	100 %	100 %	100 %
PROBIT VALUE	11	5.842	-1	-1	-1	-1
SURV. WORMS NUMBER (3W)	10	5	4	1	0	0
PERCENTAGE OF MORTALITY	0 %	50 %	60 %	90 %	100 %	100 %
PROBIT VALUE	11	5	4.746	3.718	-1	-1
SURV. WORMS NUMBER (4W)	10	3	1	2	0	0
PERCENTAGE OF MORTALITY	0 %	70 %	90 %	80 %	100 %	100 %
PROBIT VALUE	11	4.476	3.718	4.158	-1	-1

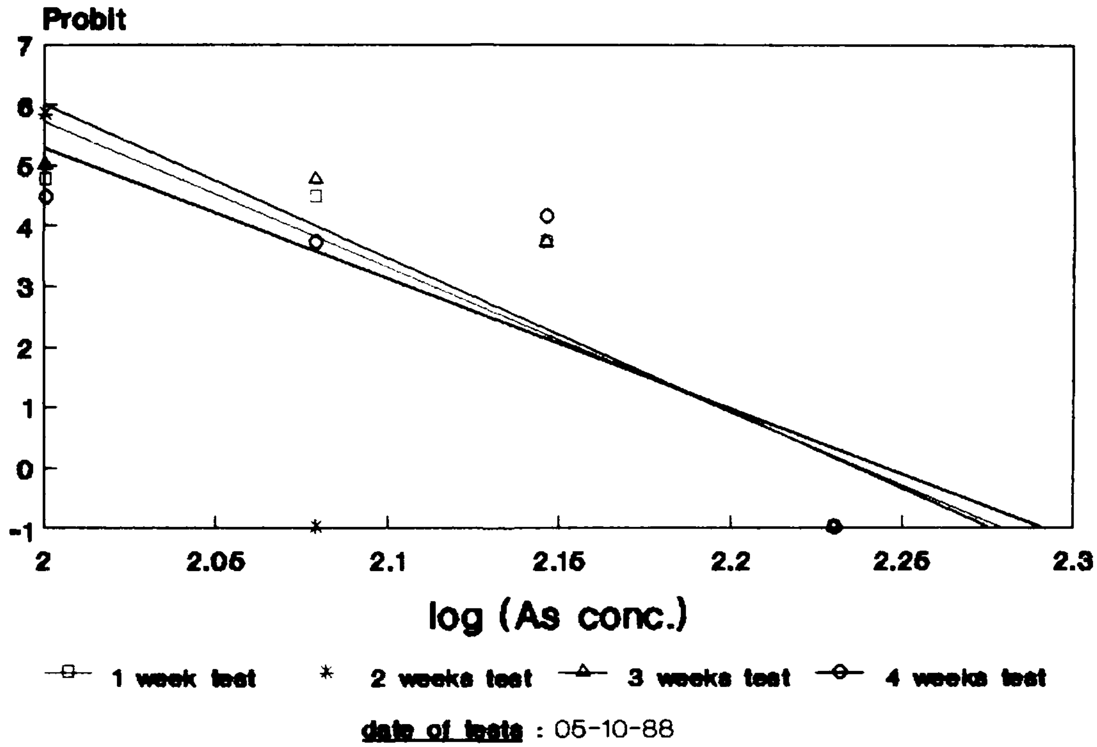
Table 10 : RESULTS OF ARSENIC TESTS OF 05-10-88 OF 1,2,3 AND 4 WEEKS LENGTHS

These results are drawn on **figure 5**.
Their analysis enable to set up another table (see below, **table 11**).

ANALYSED PARAMETERS TEST DATES	REGRESSION LINE EQUATION	CORRELAT. COEFFIC. (r)	CORRELAT. COEFFIC. TEST (t)	LC 50 IN PPM DRY WEIG. ART	LC 5 IN PPM DRY WEIG. ART
05-10-88 (1 WEEK)	Y = -24.102X+53.930 100,120,140,170 ppm	-0.876	2.565 d.of.f=2	107.182 ≈ 107	91.594 ≈ 92
05-10-88 (2 WEEKS)	Y = -86.33X+178.478 100 and 120 ppm	–	–	102.211 ≈ 102	97.824 ≈ 98
05-10-88 (3 WEEKS)	Y = -25.435X+56.880 100,120,140,170 ppm	-0.889	2.741 d.of.f=2	109.567 ≈ 110	94.407 ≈ 94
05-10-88 (4 WEEKS)	Y = -21.62X+48.537 100,120,140,170 ppm	-0.820	2.029 d.of.f=2	103.216 ≈ 103	86.628 ≈ 87

Table 11 : RESULTS ANALYSIS OF ARSENIC TESTS OF 05-10-88 OF 1,2,3 AND 4 WEEKS LENGTHS

Fig 5 : Earthworms toxicity tests
in artisol contaminated with arsenic



In the 4 tests carried out, only the second one (2 weeks length) seems doubtful because its results are different from the other three. The line is calculated with only 2 values (100 and 120 ppm), which is not very satisfying. In the other tests, we obtain correlation coefficients showing high correlation between mortality rate and medium concentration in arsenic because 0,7 < r < 0,95. This correlation is lower than for mercury tests, probably because toxic concentrations range is shorter for arsenic (from 100 to 170 ppm in As) than for mercury (36 to 1296 ppm in Hg and 80 to 640 ppm in Hg).

From t distribution table for 2 degrees of freedom, t student coefficient shows that error risk to consider there is a correlation between variables (medium concentration in arsenic and mortality rate) is about 15 %.

In spite of this high risk, we find LC50 and LC5 values very few scattered (LC50 : 107, 110, and 103 ppm ; LC5 : 92, 94 and 87 ppm - average of about 107 ppm for LC50 and 91 ppm for LC5). These values are very close because of the high slope absolute value (a) in the regression lines equations. To check these values, it would be interesting to carry out tests again in a range from 10 to 170 ppm, for instance : 10, 20, 40, 80 and 160 or 170 ppm).

c) Cadmium toxicity study

A first complementary test (07-08-87 test, 2 weeks length) has been done to find out the best range for cadmium to study (see following table 12)

* 07-08-87 test (2 weeks length)

ARTISOL CONC. IN PPM OF DRY WEIGHT	SURVIVING (N) WORMS NUMBER AFTER 14 DAYS	PERCENTAGE P OF MORTALITY P=(10-N)*10%
0 PPM	10	0 %
100 PPM	10	0 %
300 PPM	10	0 %
900 PPM	10	0 %
2700 PPM	0	100 %
8100 PPM	0	100 %

Table 12 : RESULTS OF CADMIUM TEST OF 07-08-87 (2 WEEKS LENGTH)

After this test, we made 4 others of 1 to 4 weeks length and for cadmium concentrations in Artisol of 600 to 2100 ppm, to know more precisely toxicity of this metal (see results in following table 13).

* cadmium tests

11-26-87 test : 1 week length
11-24-87 test : 2 weeks length
11-24-87 test : 3 weeks length
11-23-87 test : 4 weeks length

ARTISOL CONCENTRATION IN PPM OF DRY WEIGHT	0 PPM	600 PPM	900 PPM	1200 PPM	1500 PPM	1800 PPM	2100 PPM
log(CONC.) IN PPM	–	2.778	2.954	3.079	3.176	3.255	3.322
SURV. WORMS NUMBER (1W)	10	10	10	10	9	2	0
PERCENTAGE OF MORTALITY	0 %	0 %	0 %	0 %	10 %	80 %	100 %
PROBIT VALUE	11	11	11	11	6.282	4.158	-1
SURV. WORMS NUMBER (2W)	10	10	10	8	8	1	0
PERCENTAGE OF MORTALITY	0 %	0 %	0 %	20 %	20 %	90 %	100 %
PROBIT VALUE	11	11	11	5.842	5.842	3.718	-1
SURV. WORMS NUMBER (3W)	10	10	10	6	0	2	0
PERCENTAGE OF MORTALITY	0 %	0 %	0 %	40 %	100 %	80 %	100 %
PROBIT VALUE	11	11	11	5.253	-1	4.158	-1
SURV. WORMS NUMBER (4W)	10	10	5	8	1	1	1
PERCENTAGE OF MORTALITY	0 %	0 %	50 %	20 %	90 %	90 %	90 %
PROBIT VALUE	11	11	5	5.842	3.718	3.718	3.718

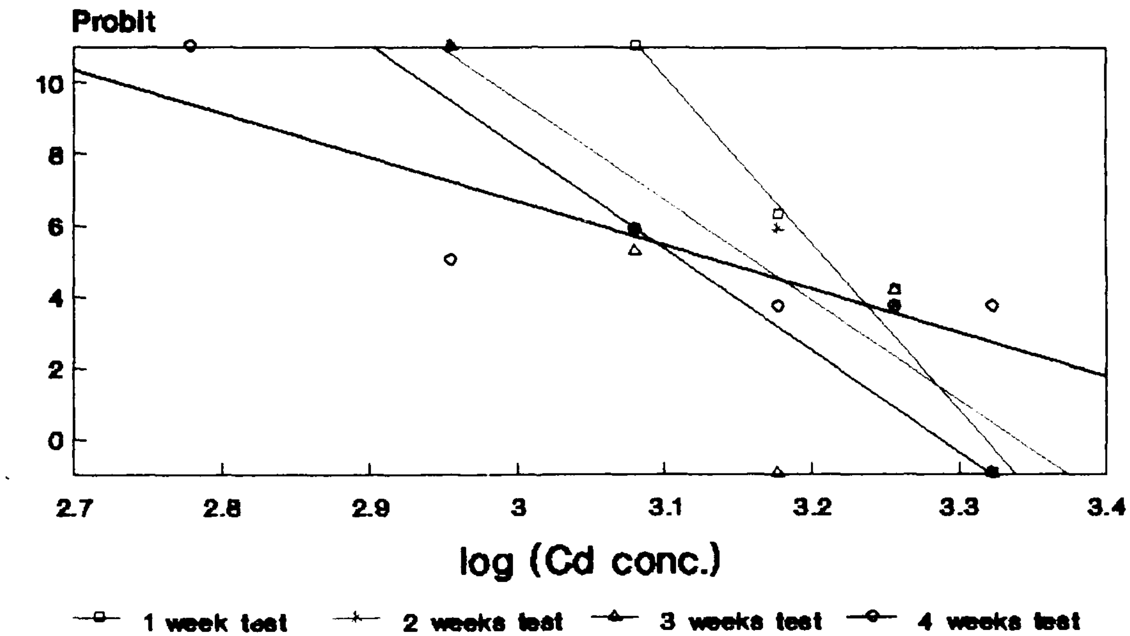
Table 13 : RESULTS OF CADMIUM TESTS OF 11-87 (23,24 & 26)
OF 1,2,3 AND 4 WEEKS LENGTHS

Figure 6 has been drawn from this table.
Result analysis is shown in following table (table 14).

ANALYSED PARAMETERS TEST DATES	REGRESSION LINE EQUATION	CORRELAT. COEFFIC. (r)	CORRELAT. COEFFIC. TEST (t)	LC 50 IN PPM DRY WEIG. ART	LC 5 IN PPM DRY WEIG. ART
11-26-87 (1 WEEK)	Y = -46.809X+155.272 1200,1500...2100 ppm	-0.985	7.940 d.of.f=2	1623.12 ≈ 1623	1496.95 ≈ 1497
11-24-87 (2 WEEKS)	Y = -28.212X+94.150 900,1200...2100 ppm	-0.947	5.089 d.of.f=3	1445.61 ≈ 1446	1263.98 ≈ 1264
11-24-87 (3 WEEKS)	Y = -28.680X+94.229 900,1200...2100 ppm	-0.833	2.610 d.of.f=3	1291.97 ≈ 1292	1132.12 ≈ 1132
11-23-87 (4 WEEKS)	Y = -12.297X+43.547 600,900...2100 ppm	-0.877	3.656 d.of.f=4	1363.34 ≈ 1363	1001.93 ≈ 1002

Table 14 : RESULTS ANALYSIS OF CADMIUM TESTS OF 11-87 (23,24 & 26) OF 1,2,3 AND 4 WEEKS LENGTHS

Fig 6 : Earthworms toxicity tests
in artisol contaminated with cadmium



date of 1 week test : 11-26-87
date of 4 weeks test : 11-23-87
date of 2 & 3 weeks tests : 11-24-87

Correlation coefficients express a very high correlation between medium concentration in cadmium and percentage of earthworms mortality, for 1 and 2 weeks length tests because r is near 0,95 or superior, and a high correlation for the 2 other tests (3 and 4 weeks length) because $0{,}70 < r < 0{,}95$.

Significant points are obtained by t values. We can find them with t distribution table for corresponding degrees of freedom (see **Annex 3**). For 1,2 and 4 weeks length tests significant point is between 1 and 2,5 % which is good, whereas for 3 weeks length test, it is between 5 and 10 %, which is not so good level because it is greater than 5 %. Cadmium LC50 is probably near 1400 ppm or in any case between 1400 and 1500 ppm. The regression lines slopes being important, there will not be a great difference between LC50 and LC5. It is really what we notice in previous table.

d) Copper toxicity study

Complementary tests are necessary to know copper toxicity (**tables 15 and 17**).

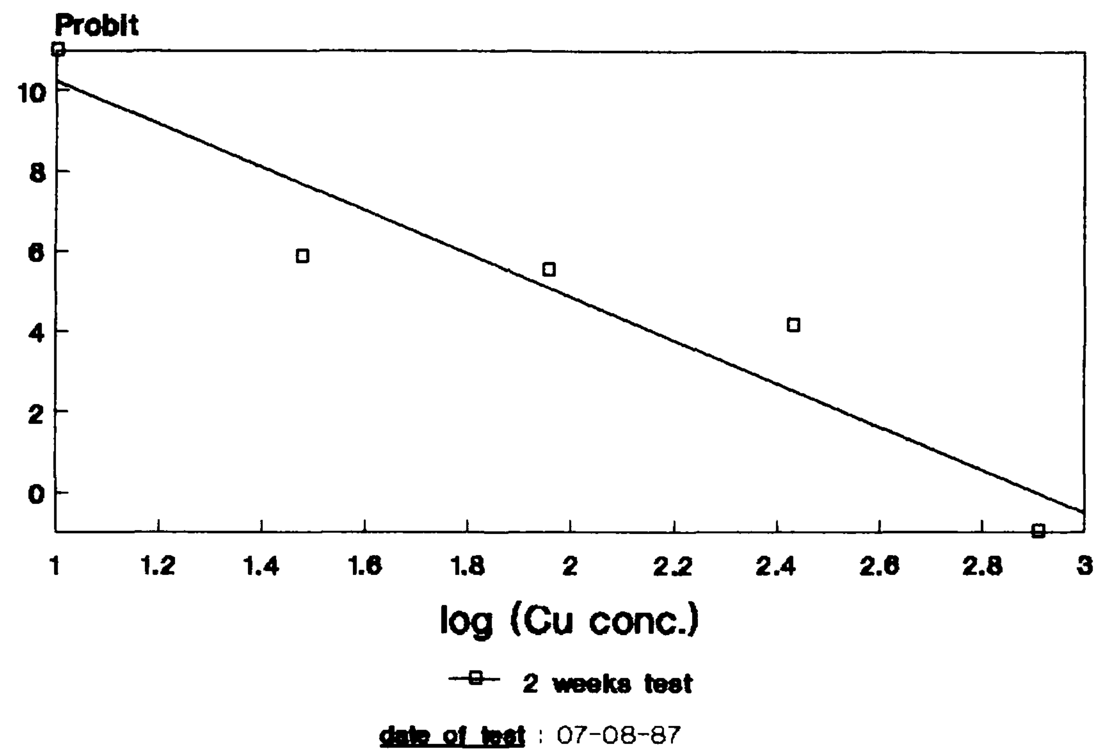
* 07-08-87 test (2 weeks length)

ARTISOL CONC. IN PPM OF DRY WEIGHT	log OF ARTISOL CONC. IN PPM OF DRY WEIGHT	SURVIVING (N) WORMS NUMBER AFTER 14 DAYS	PERCENTAGE P OF MORTALITY P=(10-N)*10%	PROBIT VALUE
0 PPM	–	10	0 %	11
10 PPM	1 PPM	10	0 %	11
30 PPM	1.477 PPM	8	20 %	5.842
90 PPM	1.954 PPM	7	30 %	5.524
270 PPM	2.431 PPM	2	80 %	4.158
810 PPM	2.908 PPM	0	100 %	-1

Table 15 : RESULTS OF COPPER TEST OF 07-08-87 (2 WEEKS LENGTH)

These results are used to draw regression line (see **figure 7**) which characteristics are given in table below (**table 16**).

Fig 7 : Earthworms toxicity tests
in artisol contaminated with copper



REGRESSION LINE EQUATION	$Y = -5.384\ X + 15.626$ with 10,30,90,270,810 ppm
CORRELATION COEFFICIENT (r)	$r = -0.946$
CORRELATION (n=5) COEFFICIENT TEST	$t = (\lvert r \rvert \sqrt{n-2})/(\sqrt{1-r^2})$ $t = 5.079$ (d.of.f=n-2=3)
LC 50 IN PPM OF DRY WEIGHT ARTISOL	94.07 PPM ≈ 94 PPM
LC 5 IN PPM OF DRY WEIGHT ARTISOL	46.55 PPM ≈ 47 PPM

Table 16 : RESULTS ANALYSIS OF COPPER TEST OF 07-08-87 (2 WEEKS LENGTH)

Correlation coefficient is close to - 0.95. Thus, we can consider the correlation is very high. Moreover, we find with t distribution table for 3 degreees of freedom (see **annex** 3) that significant point is between 1 and 2.5. %. Besides we can notice that LC5 (47 ppm) is exactly half LC50 (94 ppm).

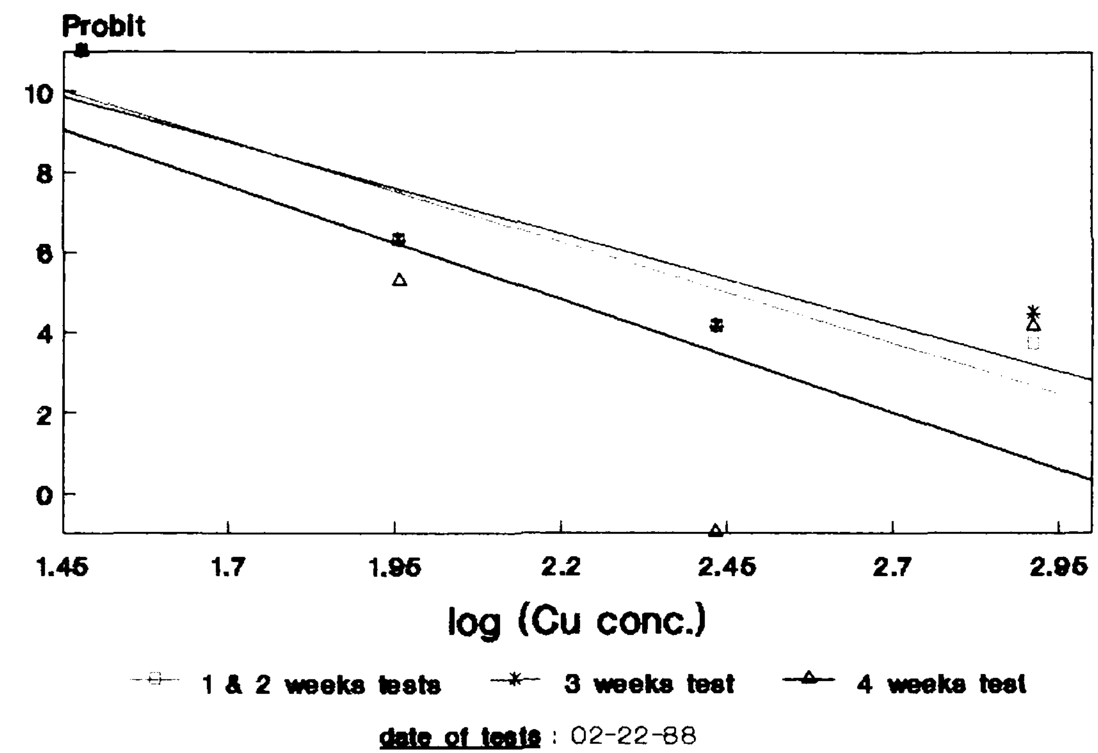
* 02-22-88 tests (1, 2, 3 and 4 weeks length)

We carried out previous test again (same concentrations) for different lengths, to study time influence on earthworm mortality (**table** 17).

ARTISOL CONCENTRATION IN PPM OF DRY WEIGHT	0 PPM	10 PPM	30 PPM	90 PPM	270 PPM	810 PPM
log(CONC.) IN PPM	–	1	1.477	1.954	2.431	2.908
SURV. WORMS NUMBER (1W)	10	10	10	9	2	1
PERCENTAGE OF MORTALITY	0 %	0 %	0 %	10 %	80 %	90 %
PROBIT VALUE	11	11	11	6.282	4.158	3.718
SURV. WORMS NUMBER (2W)	10	10	10	9	2	1
PERCENTAGE OF MORTALITY	0 %	0 %	0 %	10 %	80 %	90 %
PROBIT VALUE	11	11	11	6.282	4.178	3.718
SURV. WORMS NUMBER (3W)	10	10	10	9	2	3
PERCENTAGE OF MORTALITY	0 %	0 %	0 %	10 %	80 %	70 %
PROBIT VALUE	11	11	11	6.282	4.158	4.476
SURV. WORMS NUMBER (4W)	10	10	10	6	0	2
PERCENTAGE OF MORTALITY	0 %	0 %	0 %	40 %	100 %	80 %
PROBIT VALUE	11	11	11	5.253	-1	4.158

Table 17 : RESULTS OF COPPER TEST OF 02-22-88 OF 1,2,3 AND 4 WEEKS LENGTHS

Fig 8 : Earthworms toxicity tests
in artisol contaminated with copper



Data has been drawn on **figure 8**.
Their analysis give following results (**table 18**).

ANALYSED PARAMETERS TEST DATES	REGRESSION LINE EQUATION	CORRELAT. COEFFIC. (r)	CORRELAT. COEFFIC. TEST (t)	LC 50 IN PPM DRY WEIG. ART	LC 5 IN PPM DRY WEIG. ART
02-22-88 (1 WEEK)	Y = -5.025X+17.307 30,90,270 & 810 ppm	-0.928	3.528 d.of.f=2	281.26 ≈ 281	132.36 ≈ 132
02-22-88 (2 WEEKS)	Y = -5.025X+17.307 30,90,270 & 810 ppm	-0.928	3.528 d.of.f=2	281.26 ≈ 281	132.36 ≈ 132
02-22-88 (3 WEEKS)	Y = -4.548X+16.451 30,90,270 & 810 ppm	-0.888	2.724 d.of.f=2	329.36 ≈ 329	143.22 ≈ 143
02-22-88 (4 WEEKS)	Y = -5.614X+17.162 30,90,270 & 810 ppm	-0.702	1.395 d.of.f=2	146.65 ≈ 147	74.69 ≈ 75

Table 18 : RESULTS ANALYSIS OF COPPER TESTS OF 02-22-88 OF 1,2,3 AND 4 WEEKS LENGTHS

Correlation is good for first and second tests (r = -0,93) but not as so good for the 2 others (3 and 4 weeks). In fact, the last 2 must be eliminated because their significant point is between 10 and 40 % which is not at all satisfying.

In first and second tests, a risk is between 5 and 10 %. It explains the great variability of LC50 and LC5 results (From 147 to 329 ppm for LC50 and from 75 to 132 ppm for LC5). However, we notice about the same ratio between LC5 and LC50 (LC50 = 2 LC5) than in 07-08-87 test with different values (LC50 = 281 ppm instead of 94 ppm and LC5 = 132 ppm instead of 47 ppm).

It would be necessary to carry out another test with copper, of two weeks length only because results seem not to be very significant beyond 2 weeks.

Actually, after a determined time other factors can interfere like for instance dead earthworms decomposition staying in medium during all test or maybe the lack of food in "Artisol".

III.3.3. Conclusion

The different chemicals tested : $CuCl_2$, $2H_2O$; $NaAsO_2$; $HgCl_2$ and $CdCl_2$, $2,5H_2O$ are toxic for earthworms. Results of different tests carried out are regrouped in **table 20**.

We can notice that there are several tests which significant point is superior to 5 %, what is generally considered as not significant.

For mercury, both tests are significant, then we will calculate LC50 and LC5 mean values.

On the other hand, arsenic tests are not very significant ; however, values are very few scattered and we will also calculate mean values.

At least, for copper and cadmium, we will choose the 3 tests the

most significant to calculate LC50 and LC5.
Results are presented in following table (table 19).

TOXIC ELEMENTS	LC 50 IN PPM	LC 5 IN PPM	log (LC 50) IN PPM
As	106 PPM	93 PPM	2.025 PPM
Cu	219 PPM	104 PPM	2.340 PPM
Hg	227 PPM	154 PPM	2.356 PPM
Cd	1477 PPM	1254 PPM	3.169 PPM

Table 19 : LETHAL CONCENTRATIONS 50 % AND 5 % FOR THE DIFFERENT TOXIC ELEMENTS

Toxicities represented by LC50 extend from 106 ppm to 1477 ppm.

In toxicology, we generally regroup products by toxicity class
- class I : very toxic products : log(LC50) < 1
- class II : toxic products : 1 < log(LC50) < 2
- class III : low toxic products : 2 log(LC50) < 3
- class IV : very low toxic products : log(LC50) > 3

According to this classification, none of the tested products are in class I or II. We have principally low toxic products (As, Cu and Hg) and a very low toxic product : cadmium (class IV).

If we compare LC5, classification is exactly the same that is to say arsenic is the most toxic product followed by copper and mercury, and at least cadmium. However, to confirm these conclusions, it will be necessary to carry out some tests again. Then, we could get more points to draw regression line and reduce significance level (case of arsenic for instance and copper). Moreover, with biological material, we must have a sufficient sample size if we want to get significant results. Thus, it would be necessary to repeat the same tests 4 or 5 times to have samples of 40 or 50 earthworms instead of 10 in the tests we carried out. It has not been possible because of lack of time and material. Thus, it would be necessary to continue experiments. Besides, it seems not to be useful to prolonge test for a month to get lethal concentrations (LC50 and LC5) and 14 days length is probably sufficient to appreciate acute toxicity of a product, which also represents time safe.

IV. METAL ANALYSIS IN EARTHWORMS

After toxicity tests described previously, we have analysed metals absorbed by earthworms during tests. For technical and economical reasons, discussed before, we have limited analysis to cadmium and copper only. We will firstly present experimental results and we will then discuss them for cadmium and copper.

IV.1. Results presentation

Results are regrouped in this report in **annex 4**. They are divided into 2 parts :
- weight data,
- concentration data for cadmium and copper.

Table 20 : RECAPITULATIVE TABLE OF RESULTS
OF TOXICITY TESTS (Hg,As,Cd,Cu)

TOXIC ELEMENTS	TESTS DATES	TESTS LENGTHS	LC 50 IN PPM	LC 5 IN PPM	SIGNIFICANCE LEVEL OF TESTS
MERCURY	07-08-87	1 WEEK	211	129	1 % < α < 2.5 %
MERCURY	09-02-87	4 WEEKS	243	179	2.5 % < α < 5 %
ARSENIC	05-10-88	1 WEEK	107	92	10 % < α < 20 %
ARSENIC	05-10-88	2 WEEKS	102	98	——
ARSENIC	05-10-88	3 WEEKS	110	94	10 % < α < 20 %
ARSENIC	05-10-88	4 WEEKS	103	87	α ≈ 10 %
CADMIUM	11-26-87	1 WEEK	1623	1497	1 % < α < 2.5 %
CADMIUM	11-24-87	2 WEEKS	1446	1264	1 % < α < 2.5 %
CADMIUM	11-24-87	3 WEEKS	1292	1132	5 % < α < 10 %
CADMIUM	11-23-87	4 WEEKS	1363	1002	1 % < α < 2.5 %
COPPER	07-08-87	2 WEEKS	94	47	1 % < α < 2.5 %
COPPER	02-22-88	1 WEEK	281	132	5 % < α < 10 %
COPPER	02-22-88	2 WEEKS	281	132	5 % < α < 10 %
COPPER	02-22-88	3 WEEKS	329	143	10 % < α < 20 %
COPPER	02-22-88	4 WEEKS	147	75	10 % < α < 20 %

They are classed by date and test length. In each series, samples are numbered (with 10 characters number).

Right part of the number (behind point) indicates contaminant box from which sample comes (for instance, Cd900 means that this sample is issued from contaminated box with 900 ppm of cadmium. When this part presents the French word "blanc" the sample comes from an "uncontaminated box" which is actually the reference. The word "stock" indicates that samples are coming from background earthworm stock. This is the reason why test length mentionned for these samples is 0 week).

The left number part (before point) is used to distinguish different samples coming from the same box (V for earthworms and 01, 02, 03 for the different samples).

Each sample contains in general 2 earthworms for analytical reasons. In fact, sample weight must be high enough. Only surviving earthworms in toxicity tests have been analysed except for a few (1 week test of 02-22-88) because dead earthworms are generally decomposed or half decomposed to be taken off the medium. Some samples contain only one earthworm when it is the only surviving or when it is a very big one. They are annotate with a special sign (a star) near their numbers. Others are composed of 3 earthworms (special sign : 0). In all other cases (not mentioned) there are 2 earthworms in each sample.

IV.2. Weight data analysis

For each sample, we find out wet and dry weights and the percentage in water.

Results analysis is shown in **table 21**.

For each series, and for the 3 kinds of data (wet weights, dry weights and percentage in water), we have calculated means, standard-deviations and confidence intervals.

Results have been devided by earthworms total number in the serie (n).

Means has been calculated as follow

For dry and wet weights $\overline{X} = \dfrac{\sum_i \text{dry or wet weight (in mg)}}{n}$

For percentage in water $\overline{X}_p = \dfrac{\sum_i n_i \, p_i}{n}$ (%)

with :

n_i : earthworms number in sample i

$n = \quad n_i$

p_i : percentage in water in sample n_i

Standard deviation is represented by Sx or Sxp

$$Sx = \sqrt{\frac{\Sigma (X - \overline{X})^2}{n}} \qquad Sxp = \sqrt{\frac{\Sigma (Xp - \overline{X})^2}{n}}$$

Confidence interval of mean is given by

Table 21 : WEIGHT SERIES STATISTICS

DIFFERENT TEST SERIES	WORMS NUMBER IN SERIES	WET WEIGHT (MG)			DRY WEIGHT (MG)			PERCENTAGE IN WATER		
		X MEAN	ST.DEV.	X1 < X MEAN < X2	X MEAN	ST.DEV.	X1 < X MEAN < X2	X MEAN	ST.DEV.	X1 < X MEAN < X2
02-26-88/0W	50	275.60	51.57	261.02 - 290.20	44.58	8.45	42.19 - 46.97	83.81	0.74	83.60 - 84.02
07-08-87/2W	43	292.70	45.35	278.87 - 306.53	49.25	9.13	46.47 - 52.03	83.19	1.63	82.69 - 83.69
11-26-87/1W	51	296.35	51.19	282.01 - 310.69	50.65	8.19	48.36 - 52.94	82.83	1.36	82.45 - 83.21
11-24-87/2W	47	258.18	42.78	245.70 - 270.66	40.24	6.87	38.24 - 42.24	84.43	1.07	84.12 - 84.74
11-24-87/3W	38	248.34	34.53	237.14 - 259.54	37.88	5.38	36.13 - 39.63	84.72	0.86	84.44 - 85.00
11-23-88/4W	37	234.55	55.52	216.30 - 252.80	34.06	8.63	31.22 - 36.90	85.39	1.55	84.88 - 85.90
02-22-88/1W	42	299.12	52.64	282.87 - 315.37	49.26	9.13	46.44 -52.08	83.53	0.94	83.24 - 83.82
02-22-88/2W	42	224.97	93.56	199.18 - 250.76	35.09	12.42	31.25 - 38.91	84.22	1.63	83.70 - 84.74
02-22-88/3W	44	257.35	117.36	221.96 - 292.74	41.18	16.80	39.11 - 46.25	83.69	1.23	83.32 - 84.06
02-22-88/4W	38	256.56	93.70	196.16 - 256.96	32.86	12.96	28.66 - 37.06	85.24	1.73	84.68 - 85.80

$$x_1 \leqslant X \leqslant x_2$$

$$\overline{X} - \frac{Sx\ t_{1-\alpha/2}}{\sqrt{n}} \leqslant \overline{X} \leqslant \overline{X} + \frac{Sx\ t_{1-\alpha/2}}{\sqrt{n}}$$

where $t_1 - \ /2$ represents variable given by student table for n-1 degrees of freedom as

$$\text{prob}\left[|t| > t_1 - \alpha/2\right] = \alpha$$

if we refer to t distribution table in **Annex 3**, we see that if we choose a risk α of 5 %, $t_1 - \alpha/2$ value is near 2, for degrees of freedom corresponding to our series ($37 \leqslant$ d of f $\leqslant 49$). Thus we took the same value (2) to calculate all confidence intervals. Results table of page shows that earthworms water content in percentage is pretty constant. Actually if we consider the whole series, mean value is between 82.83 % and 85.39 %. We find that it is near 84 % if we do average of the 10 Xp values, with a standard-deviation of about 1.3 %. Taking into account the small inaccuracy resulting from manipulation (mucus losts) this result indicates a good water control in Artisol. This table also shows a variability of wet and dry weights between the different series. This one is maybe due to the lost of earthworms weight during tests in consequence of the lack of food in medium.

However we have not got precisely weight data before tests. Then, we cannot conclude and there is probably also a variability because of series difference (for instance : series 02-22-88 (2 weeks) and 02-22-88 (3 weeks) ; mean weight after 2 weeks test is lower than the one after 3 weeks test).

Besides earthworms from background stock have probably changed (evolution of nutritional availability in manure compost heap).

They could grow fatter or thinner : we brought dung only once in April 1987.

However, it seems that our earthworms have a wet weight between 260 mg and 315 mg and a dry qeight between 42 mg and 53 mg if we take into account series 02-26-88/0W, 11-26-87/1W and 02-22-88/1W which are mean weights of background earthworm stock at the end of february 1988 (series 0 week), after one week test with cadmium (series 11-26-87/1W) and after one week test with copper (02-22-88 series/1W).

At least we notice that standard deviation of wet and dry weights in copper tests of 02-22-88 at 2, 3 and 4 weeks is more important thant in other tests.

IV.3. Concentration data analysis

IV.3.1. Cadmium

In a first part, we will analyse cadmium cocnetration results of background earthworms stock. Then, we will study metal absorption according to medium concentration and time.

IV.3.1.1. Results analysis of background stock

Analysis has been made for 23 samples of 2 earthworms in each. They have been taken at random in our reserve : earthworms background stock.

Earthworms have been cleaned and put during 2 days in uncontaminated artisol medium similar to the blank of toxicity tests. The average of these 46 earthworms give a concentration value of about 6 ppm and a standard deviation of 4.3 ppm. Then, for a level α of 5 %, confidence interval is represented by

$$6.09 - \frac{4.31}{\sqrt{46}} t_{1-\alpha/2} < \bar{x} \leqslant 6.09 + \frac{4.31}{\sqrt{46}} t_{1-\alpha/2}$$

from student table (annex) for 45 degrees of freedom (n - 1) we find : $t_{1-0,025} = 2.014$
so
$6.09 - 1.28 < \bar{x} < 6.09 + 1.28$

Then mean cadmium concentration of earthworms background stock at the end of February 88 was about between 4 and 8 ppm. Actually, this value seems to be generally the level in media considered as uncontaminated. It would have been interesting to follow cadmium evolution in time of the background stock specially before and after each adding (for instance adding of dung).

IV.3.1.2. Study of cadmium absorption by earthworms depending on medium concentration

A) 07-08-87 test (2 weeks)

The statistical analysis of tests results is presented in following table 22.

Cd MEDIUM CONCENTRATION	NUMBER OF EARTHWORMS	MEAN [Cd] IN EARTHWORMS	STANDARD DEVIATION	CONFIDENCE INTERVAL RISK $\alpha \approx 5$ %
0 PPM	6	169 PPM	38 PPM	129.11 - 208.89
100 PPM	8	390.25 PPM	24.74 PPM	369.56 - 410.94
300 PPM	5	479.6 PPM	9.31 PPM	468.04 - 491.16
900 PPM	6	560.5 PPM	24.5 PPM	534.78 - 586.22

Table 22 : STATISTICAL ANALYSIS OF CADMIUM TEST OF 07-08-87 OF 2 WEEKS LENGTH

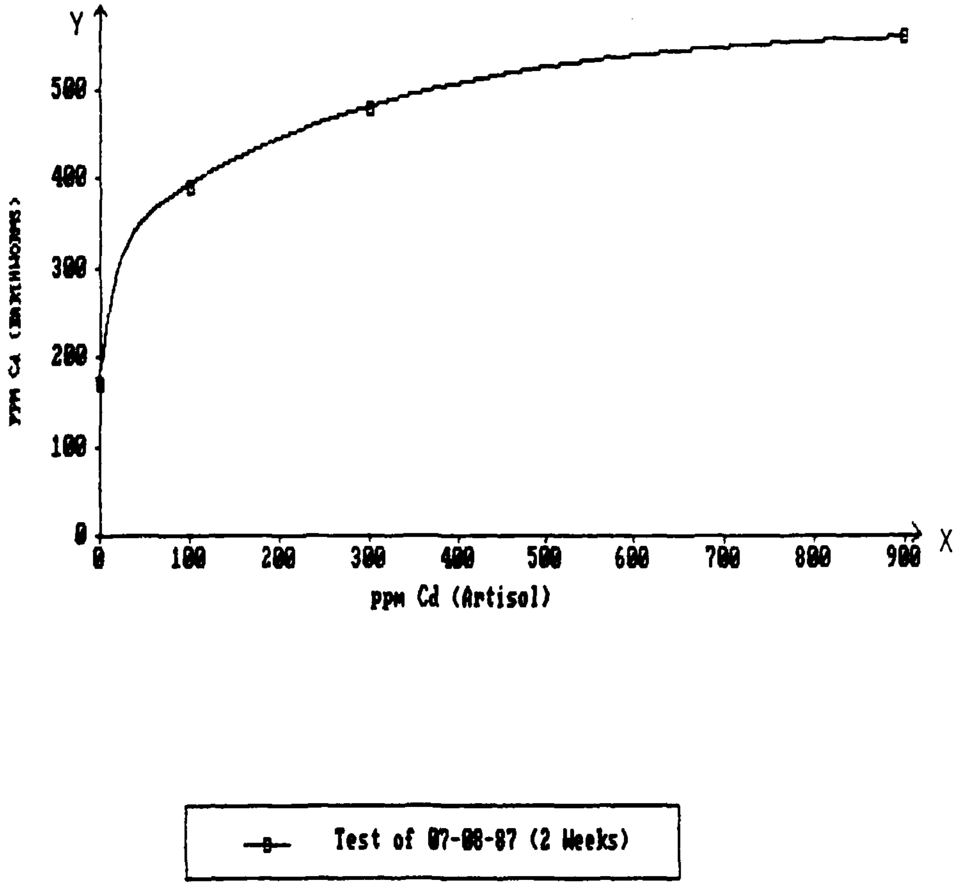
We can see that 0 cadmium level in earthworms is high over the background stock one of the end of february 88 because it is 169 ppm instead of 6 ppm. This high value is very surprising because in the same medium this figure was observed once, few time after the adding of dung. A temporary high bioavailability seems the hypothetical interpretation of this fact.

If we draw these mean results on a graph (figure 9) we notice that earthworm concentration increases, when medium concentration also increases but it reaches very quickly a step of about 600 ppm. Earthworms accumulate cadmium for small concentrations and get saturated at a determined concentration in medium (about 900 ppm). We can ajust this curve with a double exponential function : $y = k(1 - e^{ax+b} - e^{cx+d})$ (see figure 9) where k represents step value ($k \simeq 572$ ppm) towards which

FIG 9 :

Mean cadmium concentration in earthworms

FOR DIFFERENT CADMIUM CONCENTRATIONS IN "ARTISOL"



$$Y = 571.82\ (1 - E^{(-6.5\ 10^{-2}\ X\ -\ 1.304)} - E^{(-3.38\ 10^{-3}\ X\ -\ 0.837)})$$

function goes when medium concentration increases.

B) tests of 11-23, 24 and 26-87 (1, 2 3 and 4 weeks)

Analysis of these tests results is presented in **table 23**. We have also determined residual cadmium concentration in Artisol (blanck). This one is very low because its mean on 4 samples is only 3.25 ppm with a standard deviation of 0.83 ppm (*confidence interval with 5 % risk* : 1.93 ppm-4.57 ppm).

In the first series we can draw a regression line between all experimental points. We get a pretty good correlation between points : r = 0.93. Line equation obtained is : y = 0.38x - 9.47
x represents artisol concentration in ppm and y earthworm concentration.

We have eliminated V01Cd1500 sample value because it is very different of other sample values.

This earthworm was very swelled out and was certainly not in a biological condition similar to other samples.

Because of the great results dispersion in some tests, specially in 2 and 4 weeks tests, we made a variance analysis. It has been carried out with the four tests results and media concentrations from 600 to 2100 ppm (see **table 24**).

We notice in observing F_A values in table 24 that they are superior to those given by Snedecor table (see **Annex 2**) in the 1 and 2 weeks tests and inferior in the 3 and 4 weeks tests. It means in the first case (F_A < Fp (ν_A, ν_R) that medium concentration has an influence on results whereas it has not in the second case (F_A > Fp (ν_A, ν_R) for probability level chosen : 99 %.

Error risk, i.e. to accept the absence of relation between media concentrations from 600 to 2100 ppm and earthworms concentrations in 3 and 4 weeks tests whereas it does exist, is inferior to 1 %.

We can say, with a good probability that, from 3 weeks test, earthworms have the same concentration, whatever are media between 600 and 2100 ppm.

It seems normal because in media of lesser concentration earthworms could accumulate after a determined time (> 2 weeks) the same cadmium quantity than in more concentrated media.

This explains why relation is approximately linear the first week then curve has a greater slope the second, third and fourth weeks to approach quickly a step (see **figure 10**). We adjust 2nd, 3rd and 4th weeks curves with double exponentials which confirm previous results indeed for the 3rd and 4th weeks because we get the step from very low concentrations (from 50 ppm). Anyway, these conclusions would be necessary to verify.

For this, we should have prepared media of concentrations between 0 and 600 ppm in 3 and 4 weeks tests in order to see if extrapolation with calculated curve between 0 and 600 ppm, is correct.

IV.3.1.3. Study of cadmium absorption by earthwomrs depending on time

We took back tests data of 11/23-24 and 26/87 to draw a graph for different times (mean values and confidence interval in ppm, in brackets) (**table 25**).

Table 23 : STATISTICAL ANALYSIS OF RESULTS OF CADMIUM TESTS (11-23,24 & 26-87) OF 1,2,3 AND 4 WEEKS LENGTHS

SERIES	0 PPM	600 PPM	900 PPM	1200 PPM	1500 PPM	1800 PPM	2100 PPM
11-26-87/1W EARTH.NUMB(N)	10	10	10	8	8	2	—
X MEAN	1.60	246.40	335	309.25	599.50	853	—
STAND.DEV.	1.50	37.78	23.39	30.30	60.95	—	—
X1<X MEAN<X2	0.53 2.67	219.40 273.40	313.98 356.02	283.91 334.59	0 650.46	— —	— —
Σ X	16	2464	3350	2474	4796	1706	—
Σ X^2	48	621404	1130886	772430	2904920	1455218	—
11-24-87/2W EARTH.NUMB(N)	10	10	10	8	8	1	—
X MEAN	5.40	390.60	516.60	507.50	491	448	—
STAND.DEV.	0.80	56.89	103.83	119.23	166.37	—	—
X1<X MEAN<X2	4.83 5.97	349.91 431.29	442.33 590.87	407.80 607.19	351.89 630.11	— —	— —
Σ X	54	3906	5166	4060	3928	896	—
Σ X^2	298	1558050	2776554	2174172	2150068	401408	—
11-24-87/3W EARTH.NUMB(N)	10	10	10	6	—	2	—
X MEAN	14	479.80	469.20	466.67	—	564	—
STAND.DEV.	3.58	32.22	63.59	19.69	—	57	—
X1<X MEAN<X2	11.44 13.56	456.75 502.85	423.71 514.69	446 487.34	— —	51.88 1076.12	— —
Σ X	140	4798	4692	2800	—	1128	—
Σ X^2	2088	2312462	2241928	1308992	—	642690	—
11-23-87/4W EARTH.NUMB(N)	11	10	5	8	1	1	1
X MEAN	15	631.60	794.40	814.38	686	800	600
STAND.DEV.	4.67	108.69	16.26	124.04	—	—	—
X1<X MEAN<X2	11.86 18.14	553.85 709.35	774.21 814.59	710.66 918.10	— —	— —	— —
Σ X	165	6316	3972	6515	686	800	600
Σ X^2	2715	4107320	3156678	5428737	470596	640000	360000

Table 24 : VARIANCE ANALYSIS OF CADMIUM TESTS OF 11-87 (23,24 & 26) OF 1,2,3 & 4 WEEKS LENGTHS AND FOR 600 TO 2100 PPM SERIES

TESTS SERIES	EARTH. NUMB.(n)	$\Sigma\ (\Sigma X^2)$ (1)	$\Sigma\ ((\Sigma X)^2/n)$ (2)	$(\Sigma\ \Sigma X)^2/N$ (3)	Qt = (1) - (3)	Qa = (2) - (3)	Qr = Qt - Qa
11-26-87/1W	38	6884858	6824884.1	5756423.684	1128434.316	1068460.416	59973.9
11-24-87/2W	37	9060252	8986353.2	8713998.27	346253.73	272354.93	73898.8
11-24-87/3W	28	6506072	6446425.467	6430097.286	75974.714	16328.181	59646.533
11-23-87/4W	26	14163331	13920791.53	13722858.5	440472.5	197933.03	242539.47

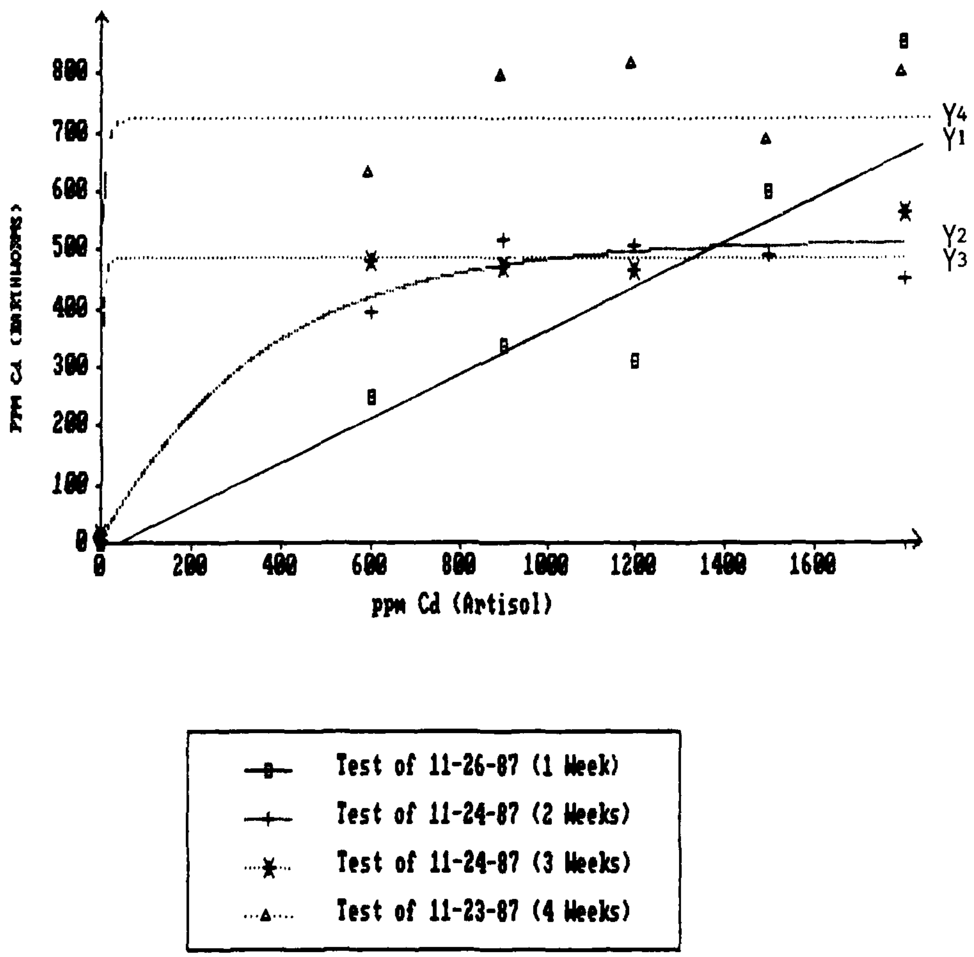
TESTS SERIES	$q_a = \frac{Q_a}{\nu_a}$	$q_r = \frac{Q_r}{\nu_r}$	$F_a = \frac{q_a}{q_r}$	$F_{1-\alpha}(\nu_a,\nu_r)$	CONCLUSIONS
11-26-87 1 WEEK	267115.104 = 4	1817.391 = 33	146.98	$F_{0.99}(4;33) \approx 4.02$	Fa > $F_{0.99}(\nu_a,\nu_r)$ => influence of media conc. on worms
11-24-87 2 WEEKS	68088.7325 = 4	2309.3375 = 32	29.48	$F_{0.99}(4;32) \approx 4.02$	Fa > $F_{0.99}(\nu_a,\nu_r)$ => influence of media conc. on worms
11-24-87 3 WEEKS	5442.727 = 3	2485.272 = 24	2.19	$F_{0.99}(3;24) \approx 4.72$	Fa < $F_{0.99}(\nu_a,\nu_r)$ => absence of infl. of media conc. on worms
11-23-87 4 WEEKS	39586.606 = 5	12126.9735 = 20	3.26	$F_{0.99}(5;20) \approx 4.10$	Fa < $F_{0.99}(\nu_a,\nu_r)$ => absence of infl. of media conc. on worms

ν_a = number of series - 1 ν_r = N - number of series N = Σ n for each series

FIG 10 :

Mean cadmium concentration in earthworms

FOR DIFFERENT CADMIUM CONCENTRATIONS IN "ARTISOL"



$$Y_1 = 0.38\ X - 9.47$$

$$Y_2 = 515.55\ (1 - E^{(-0.202\ X\ -\ 13.26)} - E^{(-2.765\ 10^{-3}\ X\ -\ 7.344\ 10^{-3})})$$

$$Y_3 = 484.87\ (1 - E^{(-0.19\ X\ -\ 0.22)} - E^{(-0.967\ X\ -\ 1.78)})$$

$$Y_4 = 721.75\ (1 - E^{(-0.114\ X\ -\ 0.664)} - E^{(-0.502\ X\ -\ 0.814)})$$

TIME IN WEEK ARTISOL [Cd]	0 WEEK	1 WEEK	2 WEEKS	3 WEEKS	4 WEEKS
600 PPM	9 PPM (7 - 11)	246 PPM (219 - 273)	391 PPM (350 - 431)	480 PPM (457 - 503)	632 PPM (554 - 709)
900 PPM	9 PPM (7 - 11)	335 PPM (314 - 356)	517 PPM (442 - 591)	469 PPM (424 - 515)	794 PPM (774 - 815)
1200 PPM	9 PPM (7 - 11)	309 PPM (284 - 335)	508 PPM (408 - 607)	467 PPM (446 - 487)	814 PPM (711 - 918)
1500 PPM	9 PPM (7 - 11)	600 PPM (549 - 650)	491 PPM (352 - 630)	– (–)	686 PPM (–)
1800 PPM	9 PPM (7 - 11)	853 PPM (–)	448 PPM (–)	564 PPM (52 - 1076)	800 PPM (–)

Table 25 : CADMIUM CONCENTRATIONS IN EARTHWORMS FOR DIFFERENT MEDIUM CONCENTRATIONS AND TIME

Cadmium absorption by earthworms depending on time is very curious (see **figure 11**). It seems to divide into 2 events. If medium concentration is not very important (untill 1200 ppm) it appears that earthworms absorb cadmium quickly, the 2 first weeks and then we have inflexion of curves even with a little step between the second and the third weeks to increase again after the third week. For higher artisol concentrations (1500 and 1800 ppm), we get a peak after a week and then the curves join other curves at the second week.

IV.3.2. Copper

We will do as in cadmium case, the analysis of copper concentration results of background worm stock, then we will study absorption of this metal depending on medium concentration and time.

IV.3.2.1. Results analysis background stock

Analysis has been done on the same 23 samples used for cadmium. They countain 2 earthworms coming from background stock, in each sample. The average of these 46 earthwomrs gives a residual copper concentration in earthwomrs of our stock at this date (end of february 88) of 12.36 ppm with a standard deviation of 2.35 ppm.

Then, for a risk α of 5 %, confidence interval obtained is

$$12.26 - \frac{2.34}{\sqrt{46}} t_{1-\alpha/2} \leqslant \overline{X} \leqslant 12.26 + \frac{2.34}{\sqrt{46}} t_{1-\alpha/2}$$

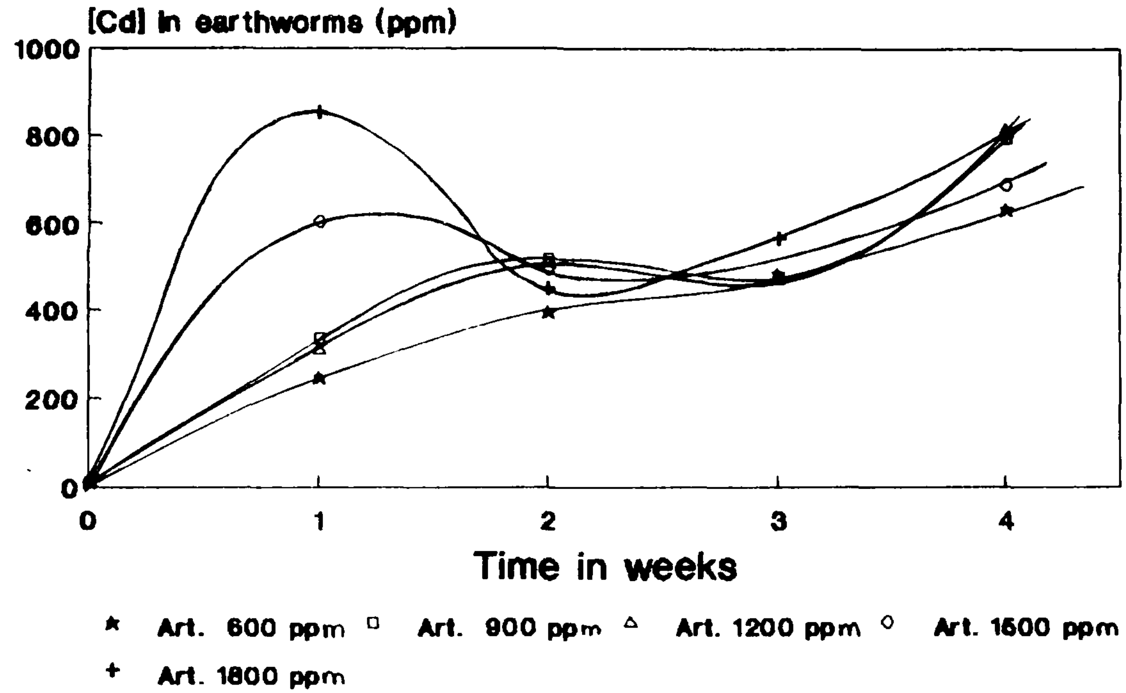
With student table (**Annex 5**) we find for 45 degrees of freedom $t_{1-0,025} = t_{0,975} = 2.014$

So :

$11.56 \text{ ppm} \leq \overline{X} \leq 12.96 \text{ ppm}$

We notice that standard deviation is smaller than in cadmium (2.35 ppm instead of 4.3 ppm) but earthworms are more concentrated in copper than in cadmium (about 12 ppm of copper and 6 ppm of cadmium). Thus, mean copper concentration of background worms stock at the end of february 1988 was approximately between 11 and 13 ppm (between 4 and 8 ppm of Cd).

Fig 11 : Absorption of cadmium by earthworms depending on time



date of tests : 11-87 (23,24 & 26)

IV.3.2.2. Study of copper absorption by earthworms depending on medium concentration

A) 07-08-87 test (2 weeks)

Statistical analysis of results is summed up in following **table 26**

Cu MEDIUM CONCENTRATION	NUMBER OF EARTHWORMS	MEAN [Cu] IN EARTHWORMS	STANDARD DEVIATION	CONFIDENCE INTERVAL RISK $\alpha \approx 5$ %
0 PPM	6	8 PPM	1 PPM	6.95 - 9.05
10 PPM	5	32.8 PPM	3.92 PPM	27.93 - 37.67
30 PPM	5	44.8 PPM	3.92 PPM	39.93 - 49.67
90 PPM	3	134 PPM	—	—
270 PPM	2	178 PPM	—	—

Table 26 : STATISTICAL ANALYSIS OF COPPER TEST OF 07-08-87 OF 2 WEEKS LENGTH

These results are drawn on a graph (figure 12)

We can again adjust the curve obtained with a double exponential function. We get a step of about 188 ppm. It is probably the concentration of earthworms saturation in copper. We notice that this value is lower than cadmium step which is about 572 ppm after the same time (2 weeks).

B) 02-22-88 test (1, 2, 3 and 4 weeks)

Statistical analysis of results is described in **table 27**. The analysis of 3 Artisol samples (blank) gives a mean residual concentration in copper of 1.67 ppm with a standard deviation of 0.47 ppm, confidence interval at 5 % level is then :

$0.5 \text{ ppm} \leq \overline{X} \leq 2.84 \text{ ppm}$

Thus copper concentration of Artisol is approximately between 0 and 3 ppm whereas it is a little higher for cadmium because we find for this metal that it is between about 2 and 5 ppm.

Mean results of 02-22-88 tests have been drawn on a graph with their confidence interval they could have been adjust with double exponential functions of the same kind as for cadmium (see **figure 13**)

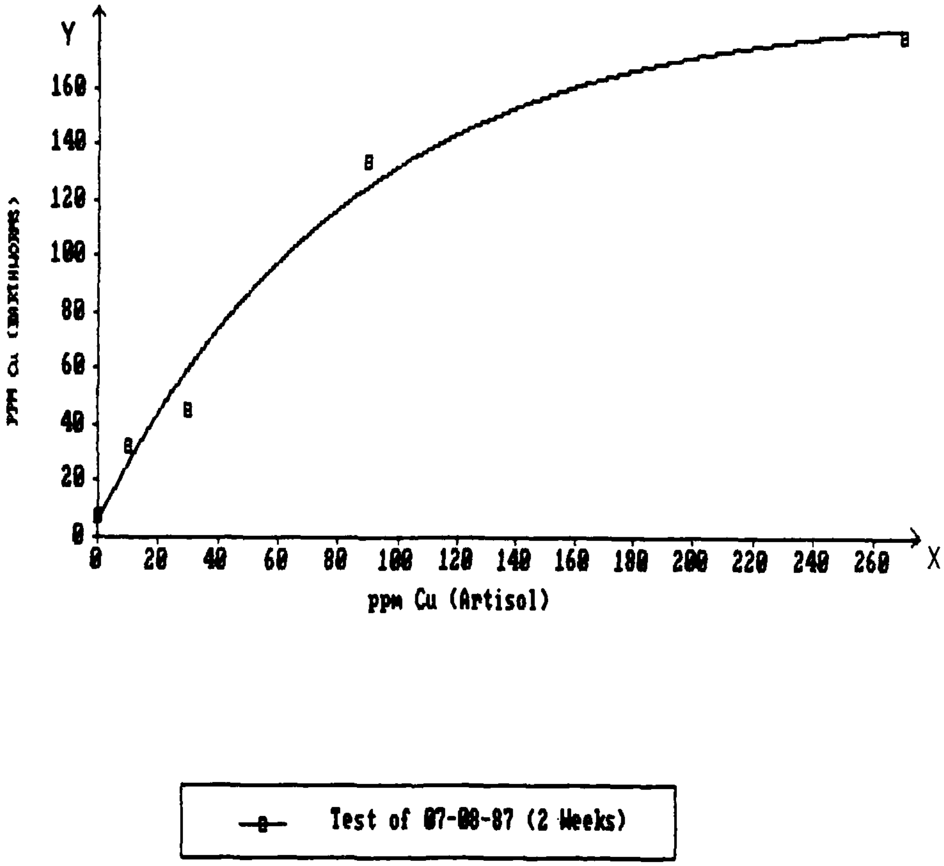
$y = k\ (1 - e^{ax+b} - e^{cx+d})$

The four constants k vary as for cadmium, with the length of test but differently. They increase between the first and the second weeks

FIG 12 :

Mean copper concentration in earthworms

FOR DIFFERENT COPPER CONCENTRATIONS IN "ARTISOL"



$$Y = 188.27\,(1 - E^{(-1.166\ 10^{-2}\ X\ -\ 3.1\ 10^{-2})} - E^{(-13.37\ X\ -\ 16.59)})$$

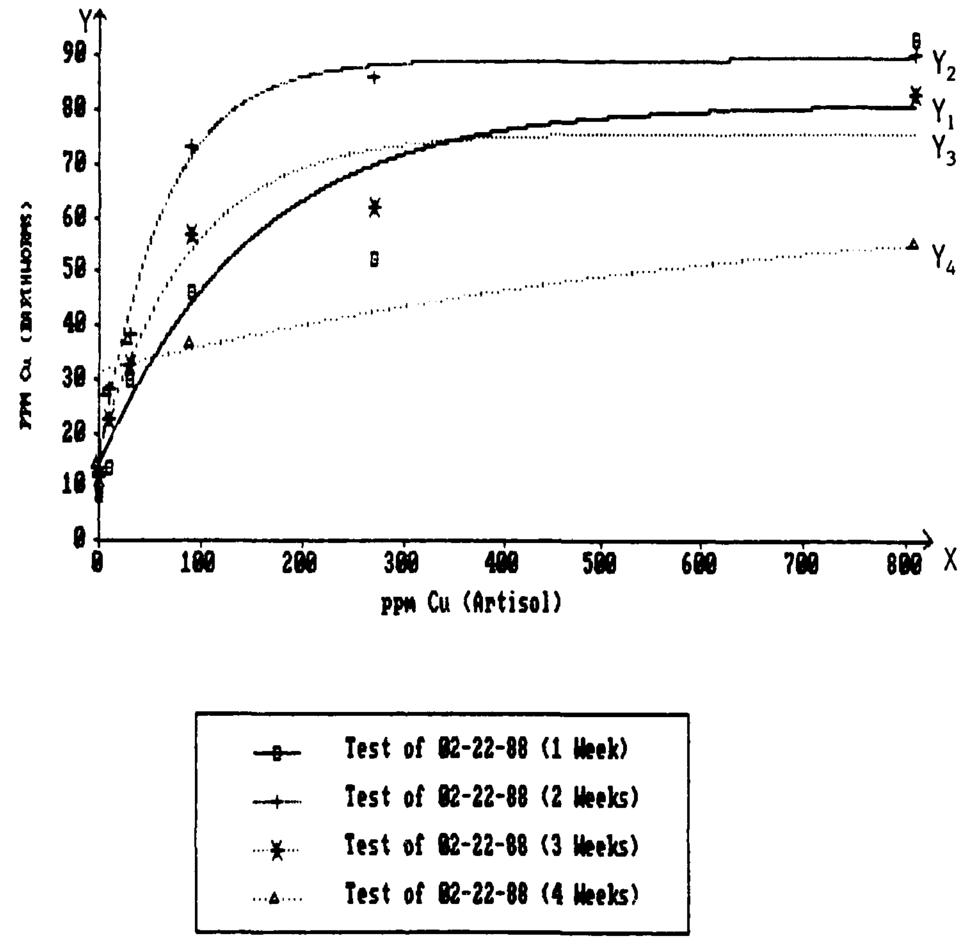
Table 27 : STATISTICAL ANALYSIS OF RESULTS OF COPPER TESTS (02-22-88) OF 1,2,3 AND 4 WEEKS LENGTHS

SERIES	0 PPM	10 PPM	30 PPM	90 PPM	270 PPM	810 PPM
02-22-88/1W EARTH.NUMB.(N)	10	10	10	9	2	1
X MEAN	8.60	13.80	30	46.22	52.50	93
STANDARD DEV.	1.50	2.04	5.22	11.54	2.50	—
X1< X MEAN <X2	7.53 9.67	12.34 15.26	26.27 33.73	37.36 55.09	30.04 74.96	— —
ΣX	86	138	300	416	105	93
ΣX^2	762	1946	9272	20426	5525	8649
02-22-88/2W EARTH.NUMB.(N)	10	10	10	9	2	1
X MEAN	12.20	28.40	38.60	73.33	86.50	90
STANDARD DEV.	3.25	4.59	3.93	9.27	13.50	—
X1< X MEAN <X2	9.88 14.52	25.12 31.68	35.79 41.41	66.20 80.46	0 208	— —
ΣX	122	284	386	660	173	90
ΣX^2	1594	8276	15054	49174	15329	8100
02-22-88/3W EARTH.NUMB.(N)	10	10	10	9	2	3
X MEAN	11.80	22.40	32.60	57	62	83
STANDARD DEV.	1.72	2.42	2.42	8.08	—	1.41
X1< X MEAN <X2	10.57 13.03	20.67 24.13	30.87 34.33	50.79 63.21	— —	79.49 86.51
ΣX	118	224	326	513	124	249
ΣX^2	1422	5076	10686	29829	7688	20673
02-22-88/4W EARTH.NUMB.(N)	10	10	10	6	—	2
X MEAN	13.90	27.40	37.40	36.50	—	55.50
STANDARD DEV.	1.58	1.62	1.62	6.50	—	3.50
X1< X MEAN <X2	12.77 15.03	26.24 28.56	36.24 38.56	29.68 43.32	— —	24.05 86.95
ΣX	139	274	374	219	—	111
ΣX^2	1957	7534	14014	8247	—	6185

Fig 13 :

Mean copper concentration in earthworms

FOR DIFFERENT CONCENTRATIONS IN "ARTISOL"



$$Y_1 = 80.92\ (1 - E^{(-6.65\ 10^{-3}\ X\ -\ 0.188)} - E^{(-29.42\ X\ -\ 2.73)})$$

$$Y_2 = 89.34\ (1 - E^{(-1.57\ 10^{-2}\ X\ -\ 0.63)} - E^{(-0.016\ X\ -\ 1.18)})$$

$$Y_3 = 75.66\ (1 - E^{(-1.11\ 10^{-2}\ X\ -\ 0.25)} - E^{(-2.77\ X\ -\ 2.41)})$$

$$Y_4 = 65\ (1 - E^{(-1.5\ 10^{-3}\ X\ -\ 0.66)} - E^{(-1.63\ X\ -\ 1.27)})$$

from about 81 to 89 ppm to decrease between the third and fourth weeks (76 ppm and 65 ppm). Such a figure suggests a metabolic detoxication.

IV.3.2.3. Study of copper absorption by earthworms depending on time

We took back data of 02-22-88 tests to analyse them vs times (mean values and confidence intervals in brackets) (see table 28).

TIME IN WEEK ARTISOL [Cu]	0 WEEK	1 WEEKS	2 WEEKS	3 WEEKS	4 WEEKS
10 PPM	12 PPM (11 - 13)	14 PPM (12 - 15)	28 PPM (25 - 32)	22 PPM (21 - 24)	27 PPM (26 - 29)
30 PPM	12 PPM (11 - 13)	30 PPM (26 - 34)	39 PPM (36 - 41)	33 PPM (31 - 34)	37 PPM (36 - 39)
90 PPM	12 PPM (11 - 13)	46 PPM (37 - 55)	73 PPM (62 - 80)	57 PPM (51 - 63)	37 PPM (30 - 43)
270 PPM	12 PPM (11 - 13)	53 PPM (30 - 75)	87 PPM (0 - 208)	52 PPM (–)	– (–)
810 PPM	12 PPM (11 - 13)	93 PPM (–)	90 PPM (–)	83 PPM (79 - 87)	56 PPM (24 - 87)

Table 28 : COPPER CONCENTRATIONS IN EARTHWORMS FOR DIFFERENT MEDIUM CONCENTRATIONS AND TIME

We notice that for small concentrations in Artisol (10 and 30 ppm) curves are about the same (see figure 14): concentration in earthworms increases to reach a maximum during the 2 first weeks and then it stays on a step after a small decrease. For higher concentrations in medium (90, 270 and 810 ppm) we also notice a peak near 2 weeks ; earthworms absorb copper quickly during the 2 first weeks, then they also release a little copper. Thus we have a decreasing of earthworms concentration the third and fourth weeks. However, the step can not be seen on graph : it is probably reached after the 4th week, because concentration in earthworms after 4 weeks has not come back to step levels of the first and second weeks (between 25 and 35 ppm).

V. CONCLUSION

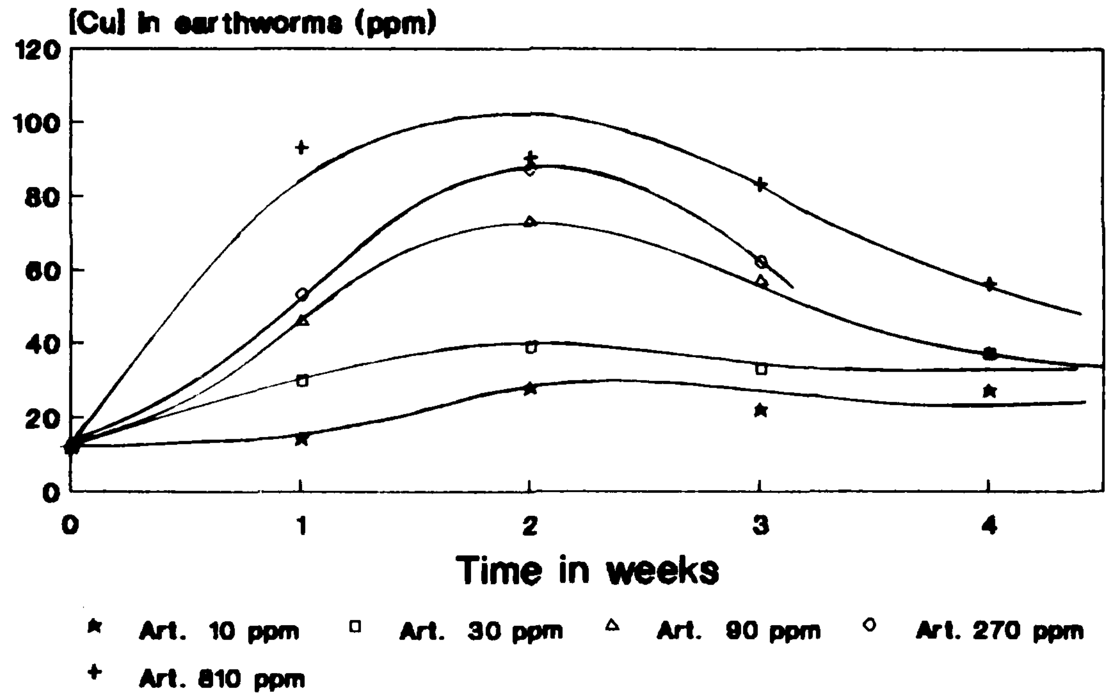
We tested on earthworms Eisenia fetida species toxicity of 4 chemicals containing respectively cadmium, copper, mercury and arsenic ($CdCl_2$, $2.5H_2O$; $CuCl_2$, $2H_2O$; $HgCl_2$ and $NaAsO_2$).

Tests has been carried out in artificial medium : "Artisol" composed of silica nearly pure according to EEC standard.

We could class chemicals tested by decreasing toxicity as follow : $NaAsO_2$; $CuCl_2,2H_2O$; $HgCl_2$ and at least $CdCl_2,2.5H_2O$. Toxicities expressed by LC50 (lethal concentrations which kill 50 % of earthworms) are 106 ppm (in As) for sodium meta-arsenite, 219 ppm (in Cu) for copper chloride dihydrate, 227 ppm (in Hg) for mercury chloride, and 1477 ppm (in Cd) for cadmium chloride 2.5 hydrate.

However for copper and arsenic, tests have been low significant. Thus, it would be necessary to confirm these conclusions to carry out

Fig 14 : Absorption of copper
by earthworms depending on time



date of tests : 02-22-88

again some tests to get more observations for LC50 determination and to decrease level of significance.

Besides, a lack of material bound us to limit tests when it would have been important to repeat 4 or 5 times the same test to get more reliable results with a population of 40 or 50 earthworms instead of 10 which are submitted to the same treatment during the same time. At least, it seems that it is not necessary to lengthen tests after 2 weeks to get LC50 to know acute toxicity of chemicals studied. We suggest in future, for the same labour to decrease test time to 14 days and then to increase number of repetition for each concentration and/or the number of concentration between the same limits.

The second part of our work has consisted in doing surviving analysis of previous tests. For material reasons, we should limit ourselves to cadmium and copper.

The absorption study of these 2 metals according to medium concentration has shown it was exponential (adjustement with a double exponential function) to increase pretty quickly towards step corresponding to saturation concentration in earthworms.

We have seen that it can change with medium concentration and even with time.

In any case, earthworms seem not to be able to survive with inner concentrations superior to 90 or 100 ppm of copper and 800 or 900 ppm of cadmium.

For copper, absorption is maximal after 2 weeks, then earthworms seem to detoxicate to come back to a lower level. Cadmium case seems more complex because even after 4 weeks, earthworms concentration is not stabilized, maybe we should have lengthened tests on longer time (5 to 10 weeks) but feeding problem would come up.

These results lead to propose a more close analysis of conditions regulating earthworm bioaccumulations (and then intoxication eventually to death) to increase our ability to interprete field data on accumulation in polluted dredged materials (or in disposal sites).

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A N N E X E S

ANNEX 1 : "Probit" values table

ANNEX 2 : SNEDECOR table (for p = 0.090)

ANNEX 3 : t distribution table

ANNEX 4 : Data
A. Weight data
B. Concentration data

ANNEX 1

"PROBIT" VALUES TABLE

% Mort.	Probit	% Mont.	Probit	% Mort.	Probit	% Mort.	Probit
0	11,0000	26	5,6433	52	4,9498	78	4,2278
1	7,3263	27	5,6128	53	4,9247	79	4,1936
2	7,0537	28	5,5826	54	4,8996	80	4,1584
3	6,8808	29	5,5534	55	4,8743	81	4,1221
4	6,7507	30	5,5244	56	4,8490	82	4,0846
5	6,6449	31	5,4959	57	4,8236	83	4,0458
6	6,5548	32	5,4677	58	4,7981	84	4,0055
7	6,4758	33	5,4399	59	4,7725	85	3,9636
8	6,4051	34	5,4125	60	4,7464	86	3,9177
9	6,3408	35	5,3853	61	4,7207	87	3,8736
10	6,2816	36	5,3585	62	4,6945	88	3,8250
11	6,2265	37	5,3319	63	4,6681	89	3,7735
12	6,1750	38	5,3055	64	4,6415	90	3,7184
13	6,1264	39	5,2793	65	4,6147	91	3,6592
14	6,0803	40	5,2533	66	4,5875	92	3,5949
15	6,0364	41	5,2275	67	4,5601	93	3,5242
16	5,9945	42	5,2019	68	4,5323	94	3,4452
17	5,9542	43	5,1764	69	4,5041	95	3,3551
18	5,9154	44	5,1510	70	4,4756	96	3,2493
19	5,8779	45	5,1257	71	4,4466	97	3,1192
20	5,8416	46	5,1004	72	4,4172	98	2,9463
21	5,8064	47	5,0753	73	4,3872	99	2,6737
22	5,7722	48	5,0502	74	4,3567	100	- 1,0000
23	5,7388	49	5,0251	75	4,3255		
24	5,7063	50	5,0000	76	4,2937		
25	5,6745	51	4,9749	77	4,2612		

ANNEX 2

SNEDECOR TABLE

For $P = 0{,}990$

ν_2 \ ν_1	1	2	3	4	5	6	7	8	9	10	ν_1 / ν_2
1	4052	4999,5	5403	5625	5764	5859	5928	5982	6022	6056	1
2	98.50	99.00	99,17	99,25	99,30	99,33	99,36	99,37	99,39	99,40	2
3	34,12	30,82	29,46	28,71	28,24	27,91	27,67	27,49	27,35	27,23	3
4	21,20	18,00	16,69	15,98	15,52	15,21	14,98	14,80	14,66	14,55	4
5	16,26	13,27	12,06	11,39	10,97	10,67	10,46	10,29	10,16	10,05	5
6	13,75	10,92	9,78	9,15	8,75	8,47	8,26	8,10	7,98	7,87	6
7	12,25	9,55	8,45	7,85	7,46	7,19	6,99	6,84	6,72	6,62	7
8	11,26	8,65	7,59	7,01	6,63	6,37	6,18	6,03	5,91	5,81	8
9	10,56	8,02	6,99	6,42	6.06	5.80	5,61	5,47	5,35	5,26	9
10	10,04	7,56	6,55	5,99	5,64	5,39	5,20	5,06	4,94	4,85	10
11	9,65	7,21	6,22	5,67	5.32	5,07	4,89	4,74	4,63	4,54	11
12	9,33	6,93	5,95	5,41	5,06	4,82	4,64	4,50	4,39	4,30	12
13	9,07	6,70	5,74	5,21	4,86	4,62	4,44	4,30	4,19	4,10	13
14	8,86	6,51	5,56	5,04	4,69	4,46	4,28	4,14	4,03	3,94	14
15	8,68	6,36	5,42	4,89	4,56	4,32	4,14	4,00	3,89	3,80	15
16	8,53	6,23	5,29	4,77	4,44	4,20	4,03	3,89	3.78	3,69	16
17	8,40	6,11	5,18	6,67	4,34	4,10	3,93	3,79	3,68	3,59	17
18	8,29	6,01	5,09	4,58	4,25	4,01	3,84	3,71	3,60	3,51	18
19	8,18	5,93	5,01	4.50	4,17	3,94	3,77	3,63	3.52	3,43	19
20	8,10	5,85	4,94	4,43	4,10	3,87	3,70	3,56	3,46	3,37	20
21	8,02	5,78	4,87	4,37	4,04	3,81	3,64	3,51	3,40	3,31	21
22	7,95	5,72	4,82	4,31	3,99	3,76	3,59	3,45	3,35	3,26	22
23	7,88	5,66	4,76	4,26	3,94	3,71	3,54	3,41	3,30	3,21	23
24	7,82	5,61	4,72	4,22	3,90	3,67	3,50	3,36	3,26	3,17	24
25	7,77	5,57	4,68	4,18	3,85	3,63	3,46	3,32	3,22	3,13	25
26	7,72	5,53	4,64	4,14	3,82	3,59	3,42	3,29	3,18	3,09	26
27	7,68	5,49	4,60	4,11	3,78	3,56	3,39	3,26	3,15	3,06	27
28	7,64	5,45	4,57	4,07	3,75	3,53	3,36	3,23	3,12	3,03	28
29	7,60	5,42	4,54	4,04	3,73	3,50	3,33	3,20	3,09	3,00	29
30	7,56	5,39	4,51	4,02	3,70	3,47	3,30	3,17	3,07	2,98	30
40	7,31	5,18	4,31	3,83	3,51	3,29	3,12	2,99	2,89	2,80	40
60	7,08	4,98	4,13	3,65	3,34	3,12	2,95	2,82	2,72	2,63	60
120	6,85	4,79	3,95	3,48	3,17	2,96	2,79	2,66	2,56	2,47	120
∞	6,63	4,61	3,78	3,32	3,02	2,80	2,64	2,51	2,41	2,32	∞
ν_2 / ν_1	1	2	3	4	5	6	7	8	9	10	ν_2 \ ν_1

ANNEX 3

t DISTRIBUTION TABLE

Degree of freedom	Probability to get a higher value whatever sign								
	0.500	0.400	0.200	0.100	0.050	0.025	0.010	0.005	0.001
1	1.000	1.376	3.078	6.314	12.706	25.452	63.657		
2	0.816	1.061	1.886	2.920	4.303	6.205	9.925	14.089	31.598
3	.765	0.978	1.638	2.353	3.182	4.176	5.841	7.453	12.941
4	.741	.941	1.533	2.132	2.776	3.495	4.604	5.598	8.610
5	.727	.920	1.476	2.015	2.571	3.163	4.032	4.773	6.859
6	.718	.906	1.440	1.943	2.447	2.969	3.707	4.317	5.959
7	.711	.896	1.415	1.895	2.365	2.841	3.499	4.029	5.405
8	.706	.889	1.397	1.860	2.306	2.752	3.355	3.832	5.041
9	.703	.883	1.383	1.833	2.262	2.685	3.250	3.690	4.781
10	.700	.879	1.372	1.812	2.228	2.634	3.169	3.581	4.587
11	.697	.876	1.363	1.796	2.201	2.593	3.106	3.497	4.437
12	.695	.873	1.356	1.782	2.179	2.560	3.055	3.428	4.318
13	.694	.870	1.350	1.771	2.160	2.533	3.012	3.372	4.221
14	.692	.868	1.345	1.761	2.145	2.510	2.977	3.326	[illegible]
15	.691	.866	1.341	1.753	2.131	2.490	2.947	3.286	4.073
16	.690	.865	1.337	1.746	2.120	2.473	2.921	3.252	4.015
17	.689	.863	1.333	1.740	2.110	2.458	2.898	3.222	3.965
18	.688	.862	1.330	1.734	2.101	2.445	2.878	3.197	3.922
19	.688	.861	1.328	1.729	2.093	2.433	2.861	3.174	3.883
20	.687	.860	1.325	1.725	2.086	2.423	2.845	3.153	3.850
21	.686	.859	1.323	1.721	2.080	2.414	2.831	3.135	3.819
22	.686	.858	1.321	1.717	2.074	2.406	2.819	3.119	3.792
23	.685	.858	1.319	1.714	2.069	2.398	2.807	3.104	3.767
24	.685	.857	1.318	1.711	2.064	2.391	2.797	3.090	3.745
25	.684	.856	1.316	1.708	2.060	2.385	2.787	3.078	3.725
26	.684	.856	1.315	1.706	2.056	2.379	2.779	3.067	3.707
27	.684	.855	1.314	1.703	2.052	2.373	2.771	3.056	3.690
28	.683	.855	1.313	1.701	2.048	2.368	2.763	3.047	3.674
29	.683	.854	1.311	1.699	2.045	2.364	2.756	3.038	3.659
30	.683	.854	1.310	1.697	2.042	2.360	2.750	3.030	3.646
35	.682	.852	1.306	1.690	2.030	2.342	2.724	2.996	3.591
40	.681	.851	1.303	1.684	2.021	2.329	2.704	2.971	3.551
45	.680	.850	1.301	1.680	2.014	2.319	2.690	2.952	3.520
50	.680	.849	1.299	1.676	2.008	2.310	2.678	2.937	3.496
55	.679	.849	1.297	1.673	2.004	2.304	2.669	2.925	3.476
60	.679	.848	1.296	1.671	2.000	2.299	2.660	2.915	3.460
70	.678	.847	1.294	1.667	1.994	2.290	2.648	2.899	3.435
80	.678	.847	1.293	1.665	1.989	2.284	2.638	2.887	3.416
90	.678	.846	1.291	1.662	1.986	2.279	2.631	2.878	3.402
100	.677	.846	1.290	1.661	1.982	2.276	2.625	2.871	3.390
120	.677	.845	1.289	1.658	1.980	2.270	2.617	2.860	3.373
∞	.6745	.8416	1.2816	1.6448	1.9600	2.2414	2.5758	2.8070	3.2905

ANNEX 4

A. Weight data

DATE : 02-26-88
WEIGHT OF EARTHWORMS
AND PERCENTAGE IN WATER
TEST LENGTH : 0 WEEK

SAMP RANK	SAMPLE NUMBERS	WEIGHT OF WET SAMPLES (mg)	WEIGHT OF DRY SAMPLES (mg)	PERCENTAGE IN WATER
1	V01. STOCK	556.30	86.90	84.38
2	V02. STOCK	679.70	108.90	83.98
3	V03. STOCK	523.30	81.70	84.39
4	V04. STOCK	578.50	100.10	82.70
5	V05. STOCK	655.50	98.80	84.93
6	V06. STOCK	509.50	79.60	84.38
7	V07. STOCK	626.10	102.10	83.69
8	V08. STOCK	465.30	73.90	84.12
9	V09. STOCK	410.00	63.30	84.56
10	V10. STOCK	799.90	132.10	83.49
11	V11. STOCK	596.20	89.00	85.07
12	V12. STOCK	629.00	100.00	84.10
13	V13. STOCK	616.80	94.70	84.65
14	V14. STOCK	701.00	109.70	84.35
15	V15. STOCK	584.80	95.90	83.60
16	V16. STOCK	410.50	68.10	83.41
17	V17. STOCK	572.50	94.50	83.49
18	V18. STOCK	568.90	98.90	82.62
19	V19. STOCK	379.00	62.20	83.59
20	V20. STOCK	496.20	87.20	82.43
21	V21. STOCK	327.00	52.30	84.01
22	V22. STOCK	476.30	82.60	82.66
23	V23. STOCK	487.60	79.10	83.78
24	V24. STOCK	580.40	91.30	84.27
25	V25. STOCK	550.40	96.10	82.54

DATE : 07-08-87
WEIGHT OF EARTHWORMS
AND PERCENTAGE IN WATER
TEST LENGTH : 2 WEEKS

SAMP RANK	SAMPLE NUMBERS	WEIGHT OF WET SAMPLES (mg)	WEIGHT OF DRY SAMPLES (mg)	PERCENTAGE IN WATER
1	V01. BLANC ▲	1041.80	212.80	79.57
2	V02. BLANC ▲	719.10	130.90	81.80
3	V01.Cd 100 ▲	983.35	144.35	85.32
4	V02.Cd 100 ▲	835.80	141.80	83.03
5	V03.Cd 100	342.35	47.70	86.07
6	V01.Cd 300 ▲	920.15	144.85	84.26
7	V02.Cd 300	512.90	93.60	81.75
8	V01.Cd 900 ▲	1013.10	151.80	85.02
9	V02.Cd 900 ▲	1048.70	159.80	84.76
10	V03.Cd 900 ▲	988.70	166.50	83.16
11	V01.Cu 10	527.50	87.20	83.47
12	V02.Cu 10 ▲	881.70	142.85	83.80
13	V01.Cu 30 ▲	962.95	177.75	81.54
14	V02.Cu 30	501.10	89.60	82.12
15	V01.Cu 90 ▲	828.50	146.30	82.34
16	V01.Cu 270	478.45	79.90	63.30

▲ 3 alive earthworms samples

DATE : 11-26-87
WEIGHT OF EARTHWORMS
AND PERCENTAGE IN WATER
TEST LENGTH : 1 WEEK

SAMP RANK	SAMPLE NUMBERS	WEIGHT OF WET SAMPLES (mg)	WEIGHT OF DRY SAMPLES (mg)	PERCENTAGE IN WATER
1	V01. BLANC	684.00	107.00	84.36
2	V02. BLANC	660.60	114.80	82.62
3	V03. BLANC	555.25	96.75	82.58
4	V04. BLANC	640.05	102.70	83.95
5	V05. BLANC	773.40	124.75	83.87
6	V01.Cd 600	607.40	103.40	82.98
7	V02.Cd 600	704.20	120.90	82.83
8	V03.Cd 600	650.50	116.45	82.10
9	V04.Cd 600	633.50	105.80	83.30
10	V05.Cd 600	561.90	90.10	83.97
11	V01.Cd 900	661.90	103.10	84.42
12	V02.Cd 900	540.70	96.10	82.23
13	V03.Cd 900	714.30	126.50	82.29
14	V04.Cd 900	570.90	107.80	81.12
15	V05.Cd 900	572.55	100.35	82.47
16	V01.Cd1200	536.40	94.20	82.44
17	V02.Cd1200	455.55	86.75	80.96
18	V03.Cd1200	574.50	101.80	82.28
19	V04.Cd1200	670.30	109.60	83.65
20	V05.Cd1200	389.00	64.90	83.32
21	V01.Cd1500*	264.80	28.70	89.16
22	V02.Cd1500	519.40	97.90	81.15
23	V03.Cd1500	332.20	67.40	82.36
24	V04.Cd1500	460.70	87.60	80.99
25	V05.Cd1500	549.00	101.60	81.49
26	V01.Cd1800	781.40	126.20	83.85

* 1 alive earthworm samples

DATE : 11-24-87
WEIGHT OF EARTHWORMS
AND PERCENTAGE IN WATER
TEST LENGTH : 2 WEEKS

SAMP RANK	SAMPLE NUMBERS	WEIGHT OF WET SAMPLES (mg)	WEIGHT OF DRY SAMPLES (mg)	PERCENTAGE IN WATER
1	V01. BLANC	542.80	87.50	83.88
2	V02. BLANC	481.60	75.60	84.30
3	V03. BLANC	581.10	81.90	85.91
4	V04. BLANC	529.70	86.70	83.63
5	V05. BLANC	696.80	109.60	84.27
6	V01.Cd 600	623.80	89.10	85.72
7	V02.Cd 600	572.20	83.60	85.39
8	V03.Cd 600	429.50	62.90	85.36
9	V04.Cd 600	552.00	75.10	86.39
10	V05.Cd 600	506.50	76.50	84.90
11	V01.Cd 900	419.55	70.45	83.21
12	V02.Cd 900	670.60	107.70	83.94
13	V03.Cd 900	542.95	91.25	83.19
14	V04.Cd 900	481.30	73.40	84.75
15	V05.Cd 900	550.00	79.50	85.55
16	V01.Cd1200	346.50	58.10	83.23
17	V02.Cd1200	569.30	94.30	83.44
18	V03.Cd1200	404.50	62.30	84.60
19	V04.Cd1200	411.00	64.30	84.36
20	V01.Cd1500	457.80	71.40	84.40
21	V02.Cd1500	571.40	86.80	84.81
22	V03.Cd1500	484.10	79.30	83.62
23	V04.Cd1500	524.00	97.00	81.49
24	V01.Cd1800*	185.40	27.00	85.44

* 1 alive earthworm sample

DATE : 11-24-87
WEIGHT OF EARTHWORMS
AND PERCENTAGE IN WATER
TEST LENGTH : 3 WEEKS

SAMP RANK	SAMPLE NUMBERS	WEIGHT OF WET SAMPLES (mg)	WEIGHT OF DRY SAMPLES (mg)	PERCENTAGE IN WATER
1	V01. BLANC	483.10	74.80	84.52
2	V02. BLANC	643.10	97.40	84.85
3	V03. BLANC	364.30	58.00	84.08
4	V04. BLANC	521.50	79.70	84.72
5	V05. BLANC	535.70	83.30	84.45
6	V01.Cd 600	512.70	76.90	85.00
7	V02.Cd 600	546.70	75.70	86.15
8	V03.Cd 600	507.40	71.90	85.83
9	V04.Cd 600	531.20	75.00	85.88
10	V05.Cd 600	536.90	78.20	85.44
11	V01.Cd 900	474.30	73.50	84.50
12	V02.Cd 900	482.50	80.00	83.42
13	V03.Cd 900	470.80	71.20	84.88
14	V04.Cd 900	464.20	70.40	84.83
15	V05.Cd 900	443.30	62.60	85.88
16	V01.Cd1200	587.50	91.80	84.37
17	V02.Cd1200	367.80	59.80	83.74
18	V03.Cd1200	409.20	63.90	84.38
19	V01.Cd1800*	252.20	44.50	82.36
20	V02.Cd1800*	302.40	50.80	83.20

* 1 alive earthworm samples

DATE : 11-23-87
WEIGHT OF EARTHWORMS
AND PERCENTAGE IN WATER
TEST LENGTH : 4 WEEKS

SAMP RANK	SAMPLE NUMBERS	WEIGHT OF WET SAMPLES (mg)	WEIGHT OF DRY SAMPLES (mg)	PERCENTAGE IN WATER
1	V01. BLANC	402.00	61.00	84.83
2	V02. BLANC	479.00	76.80	83.97
3	V03. BLANC	362.00	54.90	84.83
4	V04. BLANC	363.20	53.80	85.19
5	V05. BLANC	410.20	65.10	84.13
6	V06. BLANC*	247.60	35.90	85.50
7	V01.Cd 600	744.60	120.50	83.82
8	V02.Cd 600	621.20	82.60	86.70
9	V03.Cd 600	564.20	65.60	88.37
10	V04.Cd 600	455.20	69.40	84.75
11	V05.Cd 600	519.90	78.30	84.94
12	V01.Cd 900	541.50	86.70	83.99
13	V02.Cd 900*	313.00	36.60	88.31
14	V03.Cd 900	544.90	76.80	85.91
15	V01.Cd1200	395.20	51.20	87.04
16	V02.Cd1200*	122.50	23.50	80.82
17	V03.Cd1200	422.10	52.10	87.66
18	V04.Cd1200	466.90	64.90	86.10
19	V05.Cd1200*	197.40	30.40	84.60
20	V01.Cd1500*	186.80	26.60	85.76
21	V01.Cd1800*	152.40	23.80	84.38
22	V01.Cd2100*	166.50	23.70	85.77

*** 1 alive earthworm samples**

DATE : 02-22-88
WEIGHT OF EARTHWORMS
AND PERCENTAGE IN WATER
TEST LENGTH : 1 WEEK

SAMP RANK	SAMPLE NUMBERS	WEIGHT OF WET SAMPLES (mg)	WEIGHT OF DRY SAMPLES (mg)	PERCENTAGE IN WATER
1	V01. BLANC	667.90	113.60	82.99
2	V02. BLANC	742.30	115.90	84.39
3	V03. BLANC	705.10	114.10	83.82
4	V04. BLANC	471.30	73.40	84.43
5	V05. BLANC	520.40	83.20	84.01
6	V01.Cu 10	697.80	104.10	85.08
7	V02.Cu 10	512.60	83.30	83.75
8	V03.Cu 10	779.10	129.10	83.43
9	V04.Cu 10	703.20	115.40	83.59
10	V05.Cu 10	568.60	98.40	82.69
11	V01.Cu 30	774.40	129.70	83.25
12	V02.Cu 30	518.20	88.40	82.94
13	V03.Cu 30	438.30	72.70	83.41
14	V04.Cu 30	491.60	70.80	85.60
15	V05.Cu 30	544.00	87.50	83.92
16	V01.Cu 90	507.60	84.90	83.27
17	V02.Cu 90	652.00	119.80	81.63
18	V03.Cu 90	525.70	87.70	83.32
19	V04.Cu 90	598.10	108.20	81.91
20	V05.Cu 90 *	313.80	52.30	83.33
21	V01.Cu 270 *	211.60	35.90	83.03
22	V02.Cu 270 *	288.60	51.10	82.29
23	V03.Cu 270 ●	98.80	15.20	84.61
24	V01.Cu 810 *	331.00	50.00	84.89
25	V02.Cu 810 ●	258.10	46.60	81.94
26	V03.Cu 810 ●	88.10	12.60	85.70

* 1 alive earthworm samples
● 1 dead earthworm samples

DATE : 02-22-88
WEIGHT OF EARTHWORMS
AND PERCENTAGE IN WATER
TEST LENGTH : 2 WEEKS

SAMP RANK	SAMPLE NUMBERS	WEIGHT OF WET SAMPLES (mg)	WEIGHT OF DRY SAMPLES (mg)	PERCENTAGE IN WATER
1	V01. BLANC	325.60	48.60	85.07
2	V02. BLANC	304.50	47.40	84.43
3	V03. BLANC	309.90	47.70	84.61
4	V04. BLANC	314.30	48.00	84.73
5	V05. BLANC	312.60	48.30	84.55
6	V01.Cu 10	419.60	59.30	85.87
7	V02.Cu 10	484.80	75.70	84.38
8	V03.Cu 10	487.20	74.90	84.63
9	V04.Cu 10	506.20	73.50	85.48
10	V05.Cu 10	684.50	100.60	85.30
11	V01.Cu 30	657.60	96.80	85.28
12	V02.Cu 30	455.50	68.20	85.03
13	V03.Cu 30	547.50	82.90	84.86
14	V04.Cu 30	446.40	64.00	85.66
15	V05.Cu 30	461.40	69.40	84.96
16	V01.Cu 90	287.50	58.20	79.76
17	V02.Cu 90	326.90	57.50	82.41
18	V03.Cu 90	297.80	52.90	82.24
19	V04.Cu 90	441.70	67.70	84.67
20	V05.Cu 90*	598.10	91.30	84.74
21	V01.Cu 270*	298.80	60.30	79.82
22	V02.Cu 270*	163.50	33.40	79.57
23	V01.Cu 810*	316.70	46.70	85.25

* 1 alive earthworm samples

DATE : 02-22-88
WEIGHT OF EARTHWORMS
AND PERCENTAGE IN WATER
TEST LENGTH : 3 WEEKS

SAMP RANK	SAMPLE NUMBERS	WEIGHT OF WET SAMPLES (mg)	WEIGHT OF DRY SAMPLES (mg)	PERCENTAGE IN WATER
1	V01. BLANC	328.70	53.50	83.72
2	V02. BLANC	730.30	112.90	84.54
3	V03. BLANC	513.30	81.50	84.12
4	V04. BLANC	608.80	97.50	83.98
5	V05. BLANC	416.10	67.80	83.71
6	V01.Cu 10	534.70	82.80	84.51
7	V02.Cu 10	697.60	104.80	84.98
8	V03.Cu 10	672.10	109.90	83.65
9	V04.Cu 10	713.70	108.30	84.83
10	V05.Cu 10	428.70	66.50	84.49
11	V01.Cu 30	531.20	84.70	84.05
12	V02.Cu 30	437.30	68.90	84.24
13	V03.Cu 30	328.60	54.30	83.47
14	V04.Cu 30	325.00	48.30	85.14
15	V05.Cu 30	1124.00	166.00	85.23
16	V01.Cu 90	417.60	75.30	81.97
17	V02.Cu 90	310.40	58.50	81.15
18	V03.Cu 90	281.20	53.00	81.15
19	V04.Cu 90	334.40	60.90	81.79
20	V05.Cu 90 *	139.50	26.10	81.29
21	V01.Cu 270	274.20	43.30	84.21
22	V01.Cu 810 *	666.40	101.90	84.71
23	V02.Cu 810	510.10	85.10	83.32

* 1 alive earthworm samples

DATE : 02-22-88
WEIGHT OF EARTHWORMS
AND PERCENTAGE IN WATER
TEST LENGTH : 4 WEEKS

SAMP RANK	SAMPLE NUMBERS	WEIGHT OF WET SAMPLES (mg)	WEIGHT OF DRY SAMPLES (mg)	PERCENTAGE IN WATER
1	V01. BLANC	460.60	61.90	86.56
2	V02. BLANC	449.10	67.60	84.95
3	V03. BLANC *	679.40	97.90	85.59
4	V04. BLANC	486.90	67.10	86.22
5	V05. BLANC ▲	376.10	54.10	85.62
6	V01.Cu 10	459.40	58.50	87.27
7	V02.Cu 10	431.20	65.80	84.74
8	V03.Cu 10	307.80	52.80	82.85
9	V04.Cu 10	437.40	57.20	86.92
10	V05.Cu 10	354.30	51.50	85.46
11	V01.Cu 30	513.20	74.10	85.56
12	V02.Cu 30	656.50	91.70	86.03
13	V03.Cu 30	487.60	72.30	85.17
14	V04.Cu 30	485.20	65.50	86.50
15	V05.Cu 30	379.70	49.60	86.94
16	V01.Cu 90 *	309.70	44.40	85.66
17	V02.Cu 90	569.10	84.00	85.24
18	V03.Cu 90 ▲	329.90	64.50	80.45
19	V01.Cu 810 *	234.30	37.50	83.99
20	V02.Cu 810 *	201.70	30.70	84.78

* 1 alive earthworm samples
▲ 3 alive earthworms samples

ANNEX 4

B. Concentration data
   Cadmium
   Copper

DATE : 02-26-88
SAMPLE CADMIUM
CONCENTRATIONS TABLE
TEST LENGTH : 0 WEEK

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. STOCK	50	0.004	0.20	86.90	2
V02. STOCK	50	0.014	0.70	108.90	6
V03. STOCK	50	0.009	0.45	81.70	6
V04. STOCK	50	0.024	1.20	100.10	12
V05. STOCK	50	0.014	0.70	98.80	7
V06. STOCK	50	0.015	0.75	79.60	9
V07. STOCK	50	0.019	0.95	102.10	9
V08. STOCK	50	0.015	0.75	73.90	10
V09. STOCK	50	0.022	1.10	63.30	17
V10. STOCK	50	0.009	0.45	132.10	3
V11. STOCK	50	0.021	1.05	89.00	12
V12. STOCK	50	0.011	0.55	100.00	6
V13. STOCK	50	0.022	1.10	94.70	12
V14. STOCK	50	0.009	0.45	109.70	4
V15. STOCK	50	0.005	0.25	95.90	3
V16. STOCK	50	0.003	0.15	68.10	2
V17. STOCK	50	0.002	0.10	94.50	1
V18. STOCK	50	0.007	0.35	98.90	4
V19. STOCK	50	0.000	0.00	62.20	0
V21. STOCK	50	0.000	0.00	52.30	0
V23. STOCK	50	0.006	0.30	79.10	4
V24. STOCK	50	0.007	0.35	91.30	4
V25. STOCK	50	0.014	0.70	96.10	7

DATE : 07-08-87
SAMPLE CADMIUM
CONCENTRATIONS TABLE
TEST LENGTH : 2 WEEKS

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. BLANC	100	0.279	27.90	212.80	131
V02. BLANC	50	0.541	27.05	130.90	207
V01.Cd 100	50	1.091	54.55	144.35	378
V02.Cd 100	50	1.061	53.05	141.80	374
V03.Cd 100	50	0.413	20.65	47.70	433
V01.Cd 300	50	1.368	68.40	144.85	472
V02.Cd 300	50	0.919	45.95	93.60	491
V01.Cd 900	50	1.627	81.35	151.80	536
V02.Cd 900	50	1.869	93.45	159.80	585

DATE : 11-26-87
SAMPLE CADMIUM
CONCENTRATIONS TABLE
TEST LENGTH : 1 WEEK

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. BLANC	50	0.001	0.05	107.00	0
V02. BLANC	50	0.001	0.05	114.80	0
V03. BLANC	50	0.008	0.40	96.75	4
V04. BLANC	50	0.005	0.25	102.70	2
V05. BLANC	50	0.005	0.25	124.75	2
V01.Cd 600	50	0.528	26.40	103.40	255
V02.Cd 600	50	0.555	27.75	120.90	230
V03.Cd 600	50	0.491	24.55	116.45	211
V04.Cd 600	50	0.668	33.40	105.80	316
V05.Cd 600	50	0.396	19.80	90.10	220
V01.Cd 900	50	0.736	36.80	103.10	357
V02.Cd 900	50	0.623	31.15	96.10	324
V03.Cd 900	50	0.822	41.10	126.50	325
V04.Cd 900	50	0.629	31.45	107.80	292
V05.Cd 900	50	0.757	37.85	100.35	377
V01.Cd1200	50	0.536	26.80	94.20	285
V02.Cd1200	50	0.552	27.60	86.75	318
V03.Cd1200	50	0.569	28.45	101.80	279
V05.Cd1200	50	0.461	23.05	64.90	355
V01.Cd1500	25	2.046	51.15	28.70	1782
V02.Cd1500	50	1.092	54.60	97.90	558
V03.Cd1500	50	0.725	36.25	67.40	538
V04.Cd1500	50	1.061	53.05	87.60	606
V05.Cd1500	50	1.415	70.75	101.60	696
V01.Cd1800	50	2.153	107.65	126.20	853

DATE : 11-24-87
SAMPLE CADMIUM
CONCENTRATIONS TABLE
TEST LENGTH : 2 WEEKS

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. BLANC	50	0.008	0.40	87.50	5
V02. BLANC	50	0.011	0.55	75.60	7
V03. BLANC	50	0.008	0.40	81.90	5
V04. BLANC	50	0.009	0.45	86.70	5
V05. BLANC	50	0.010	0.50	109.60	5
V01.Cd 600	50	0.660	33.00	89.10	370
V02.Cd 600	50	0.762	38.10	83.60	456
V03.Cd 600	50	0.541	27.05	62.90	430
V04.Cd 600	50	0.439	21.95	75.10	292
V05.Cd 600	50	0.619	30.95	76.50	405
V01.Cd 900	50	0.744	37.20	70.45	528
V02.Cd 900	50	0.858	42.90	107.70	398
V03.Cd 900	50	0.775	38.75	91.25	425
V04.Cd 900	50	0.792	39.60	73.40	540
V05.Cd 900	50	1.100	55.00	79.50	692
V01.Cd1200	50	0.749	37.45	58.10	645
V02.Cd1200	50	0.663	33.15	94.30	352
V03.Cd1200	50	0.541	27.05	62.30	434
V04.Cd1200	50	0.770	38.50	64.30	599
V01.Cd1500	50	1.071	53.55	71.40	750
V02.Cd1500	50	0.580	29.00	86.80	334
V03.Cd1500	50	0.829	41.45	79.30	523
V04.Cd1500	50	0.692	34.60	97.00	357
V01.Cd1800	25	0.484	12.10	27.00	448

DATE : 11-24-87
SAMPLE CADMIUM
CONCENTRATIONS TABLE
TEST LENGTH : 3 WEEKS

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. BLANC	50	0.015	0.75	74.80	10
V02. BLANC	50	0.024	1.20	97.40	12
V03. BLANC	50	0.023	1.15	58.00	20
V04. BLANC	50	0.019	0.95	79.70	12
V05. BLANC	50	0.026	1.30	83.30	16
V01.Cd 600	50	0.724	36.20	76.90	471
V02.Cd 600	50	0.800	40.00	75.70	528
V03.Cd 600	50	0.711	35.55	71.90	494
V04.Cd 600	50	0.643	32.15	75.00	429
V05.Cd 600	50	0.746	37.30	78.20	477
V01.Cd 900	50	0.767	38.35	73.50	522
V02.Cd 900	50	0.652	32.60	80.00	408
V03.Cd 900	50	0.769	38.45	71.20	540
V04.Cd 900	50	0.698	34.90	70.40	496
V05.Cd 900	50	0.476	23.80	62.60	380
V01.Cd1200	50	0.815	40.75	91.80	444
V02.Cd1200	50	0.555	27.75	59.80	464
V03.Cd1200	50	0.629	31.45	63.90	492
V01.Cd1800	50	0.451	22.55	44.50	507
V02.Cd1800	50	0.631	31.55	50.80	621

DATE : 11-23-87
SAMPLE CADMIUM
CONCENTRATIONS TABLE
TEST LENGTH : 4 WEEKS

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. BLANC	50	0.022	1.10	61.00	18
V02. BLANC	50	0.013	0.65	76.80	8
V03. BLANC	50	0.019	0.95	54.90	17
V04. BLANC	50	0.022	1.10	53.60	20
V05. BLANC	50	0.013	0.65	65.10	10
V06. BLANC	25	0.027	0.68	35.90	19
V01.Cd 600	50	1.180	59.00	120.50	490
V02.Cd 600	50	1.107	55.35	92.60	570
V03.Cd 600	50	0.761	38.05	65.60	580
V04.Cd 600	50	1.133	56.65	69.40	816
V05.Cd 600	50	0.942	47.10	78.30	602
V01.Cd 900	50	1.395	69.75	86.70	804
V02.Cd 900	25	1.116	27.90	36.60	762
V03.Cd 900	50	1.230	61.50	76.80	801
V01.Cd1200	50	0.926	46.30	51.20	904
V02.Cd1200	25	0.809	20.23	23.50	861
V03.Cd1200	50	0.899	44.95	52.10	863
V04.Cd1200	50	1.053	52.65	64.90	811
V05.Cd1200	25	0.606	15.15	30.40	498
V01.Cd1500	25	0.730	18.25	26.60	686
V01.Cd1800	25	0.762	19.05	23.80	800
V01.Cd2100	25	0.569	14.23	23.70	600

DATE : 02-26-88
SAMPLE COPPER
CONCENTRATIONS TABLE
TEST LENGTH : 0 WEEK

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. STOCK	50	0.017	0.85	86.90	10
V02. STOCK	50	0.023	1.15	108.90	11
V03. STOCK	50	0.019	0.95	81.70	12
V04. STOCK	50	0.022	1.10	100.10	11
V05. STOCK	50	0.018	0.90	98.80	9
V06. STOCK	50	0.016	0.80	79.60	10
V07. STOCK	50	0.021	1.05	102.10	10
V08. STOCK	50	0.023	1.15	73.90	16
V09. STOCK	50	0.013	0.65	63.30	10
V10. STOCK	50	0.027	1.35	132.10	10
V11. STOCK	50	0.022	1.10	89.00	12
V12. STOCK	50	0.033	1.65	100.00	17
V13. STOCK	50	0.025	1.25	94.70	13
V14. STOCK	50	0.021	1.05	109.70	10
V15. STOCK	50	0.024	1.20	95.90	13
V16. STOCK	50	0.021	1.05	68.10	15
V17. STOCK	50	0.019	0.95	94.50	10
V18. STOCK	50	0.028	1.40	98.90	14
V19. STOCK	50	0.015	0.75	62.20	12
V21. STOCK	50	0.015	0.75	52.30	14
V23. STOCK	50	0.022	1.10	79.10	14
V24. STOCK	50	0.022	1.10	91.30	12
V25. STOCK	50	0.033	1.65	96.10	17

DATE : 07-08-87
SAMPLE COPPER
CONCENTRATIONS TABLE
TEST LENGTH : 2 WEEKS

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. BLANC	100	0.015	1.50	212.80	7
V02. BLANC	50	0.024	1.20	130.90	9
V01.Cu 10	50	0.048	2.40	87.20	28
V02.Cu 10	50	0.104	5.20	142.85	36
V01.Cu 30	100	0.086	8.60	177.75	48
V02.Cu 30	50	0.071	3.55	89.60	40
V01.Cu 90	50	0.393	19.65	146.30	134
V01.Cu 270	50	0.284	14.20	79.90	178

DATE : 02-22-88
SAMPLE COPPER
CONCENTRATIONS TABLE
TEST LENGTH : 1 WEEK

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. BLANC	50	0.022	1.10	113.60	10
V02. BLANC	50	0.020	1.00	115.90	9
V03. BLANC	50	0.014	0.70	114.10	6
V04. BLANC	50	0.014	0.70	73.40	10
V05. BLANC	50	0.014	0.70	83.20	8
V01.Cu 10	50	0.034	1.70	104.10	16
V02.Cu 10	50	0.023	1.15	83.30	14
V03.Cu 10	50	0.042	2.10	129.10	16
V04.Cu 10	50	0.026	1.30	115.40	11
V05.Cu 10	50	0.024	1.20	98.40	12
V01.Cu 30	50	0.053	2.65	129.70	20
V02.Cu 30	50	0.057	2.85	88.40	32
V03.Cu 30	50	0.050	2.50	72.70	34
V04.Cu 30	50	0.043	2.15	70.80	30
V05.Cu 30	50	0.060	3.00	87.50	34
V01.Cu 90	50	0.046	2.30	84.90	27
V02.Cu 90	50	0.109	5.45	119.80	45
V03.Cu 90	50	0.086	4.30	87.70	49
V04.Cu 90	50	0.129	6.45	108.20	60
V05.Cu 90	50	0.057	2.85	52.30	54
V01.Cu 270	25	0.072	1.80	35.90	50
V03.Cu 270	25	0.049	1.22	15.20	81
V01.Cu 810	50	0.093	4.65	50.00	93
V02.Cu 810	50	0.074	3.70	46.60	79
V03.Cu 810	25	0.164	4.10	12.60	325

DATE : 02-22-88
SAMPLE COPPER
CONCENTRATIONS TABLE
TEST LENGTH : 2 WEEKS

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. BLANC	50	0.015	0.75	48.60	15
V02. BLANC	50	0.016	0.80	47.40	17
V03. BLANC	50	0.009	0.45	47.70	9
V04. BLANC	50	0.009	0.45	48.00	9
V05. BLANC	50	0.011	0.55	48.30	11
V01.Cu 10	50	0.026	1.30	59.30	22
V02.Cu 10	50	0.037	1.85	75.70	24
V03.Cu 10	50	0.046	2.30	74.90	31
V04.Cu 10	50	0.046	2.30	73.50	31
V05.Cu 10	50	0.069	3.45	100.60	34
V01.Cu 30	50	0.071	3.55	96.80	37
V02.Cu 30	50	0.056	2.80	68.20	41
V03.Cu 30	50	0.057	2.85	82.90	34
V04.Cu 30	50	0.046	2.30	64.00	36
V05.Cu 30	50	0.062	3.10	69.40	45
V01.Cu 90	50	0.091	4.55	58.20	78
V02.Cu 90	50	0.084	4.20	57.50	73
V03.Cu 90	50	0.091	4.55	52.90	86
V04.Cu 90	50	0.081	4.05	67.70	60
V05.Cu 90	50	0.121	6.05	91.30	66
V01.Cu 270	50	0.088	4.40	60.30	73
V02.Cu 270	50	0.067	3.35	33.40	100
V01.Cu 810	50	0.084	4.20	46.70	90

DATE : 02-22-88
SAMPLE COPPER
CONCENTRATIONS TABLE
TEST LENGTH : 3 WEEKS

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. BLANC	50	0.012	0.60	53.50	11
V02. BLANC	50	0.025	1.25	112.90	11
V03. BLANC	50	0.020	1.00	81.50	12
V04. BLANC	50	0.029	1.45	97.50	15
V05. BLANC	50	0.014	0.70	67.80	10
V01.Cu 10	50	0.041	2.05	82.80	25
V02.Cu 10	50	0.039	1.95	104.80	19
V03.Cu 10	50	0.053	2.65	109.90	24
V04.Cu 10	50	0.043	2.15	108.30	20
V05.Cu 10	50	0.032	1.60	66.50	24
V01.Cu 30	50	0.056	2.80	84.70	33
V02.Cu 30	50	0.047	2.35	68.90	34
V03.Cu 30	50	0.038	1.90	54.30	35
V04.Cu 30	50	0.032	1.60	48.30	33
V05.Cu 30	100	0.046	4.60	166.00	28
V01.Cu 90	50	0.094	4.70	75.30	62
V02.Cu 90	50	0.054	2.70	58.50	46
V03.Cu 90	50	0.063	3.15	53.00	59
V04.Cu 90	50	0.064	3.20	60.90	53
V05.Cu 90	25	0.076	1.90	26.10	73
V01.Cu 270	50	0.054	2.70	43.30	62
V01.Cu 810	50	0.166	8.30	101.90	81
V02.Cu 810	50	0.143	7.15	85.10	84

DATE : 02-22-88
SAMPLE COPPER
CONCENTRATIONS TABLE
TEST LENGTH : 4 WEEKS

SAMPLES NUMBERS	VOLUME (in ml)	CONCENTRATION (in mg/l)	CON*VOL (ug)	DRY WEIGHT SAMP.(mg)	Mg METAL/Kg DRY WEIGHT
V01. BLANC	50	0.017	0.85	61.90	14
V02. BLANC	50	0.018	0.90	67.60	13
V03. BLANC	50	0.021	1.05	97.90	11
V04. BLANC	50	0.017	0.85	67.10	13
V05. BLANC	50	0.017	0.85	54.10	16
V01.Cu 10	50	0.033	1.65	58.50	28
V02.Cu 10	50	0.036	1.80	65.80	27
V03.Cu 10	50	0.029	1.45	52.80	27
V04.Cu 10	50	0.029	1.45	57.20	25
V05.Cu 10	50	0.031	1.55	51.50	30
V01.Cu 30	50	0.058	2.90	74.10	39
V02.Cu 30	50	0.065	3.25	91.70	35
V03.Cu 30	50	0.052	2.60	72.30	36
V04.Cu 30	50	0.051	2.55	65.50	39
V05.Cu 30	50	0.038	1.90	49.60	38
V01.Cu 90	50	0.027	1.35	44.40	30
V02.Cu 90	50	0.051	2.55	84.00	30
V03.Cu 90	50	0.055	2.75	64.50	43
V01.Cu 810	50	0.039	1.95	37.50	52
V02.Cu 810	50	0.036	1.80	30.70	59