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The Development of Toxicity Tests Using Two Marine Nematode Species.

by

Thierry Bogaert

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
M. Sc.
in
The Department of Zoology

Winnipeg, Manitoba, 1984

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Dr. K.W. Lande

Dr. T. Dandy

Dr. J. Klaverkamp

External Examiner
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Chairman
Dr. K.W. Stewart

Date March 23rd, 1984

THE DEVELOPMENT OF TOXICITY TESTS USING TWO MARINE NEMATODE SPECIES

by

Thierry Bogaert

A thesis submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

MASTER OF SCIENCE

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ABSTRACT

A simple rapid long-term, quantitative toxicity assay for marine contaminants was developed using two Monhysterid nematodes, Diplolaimelloides bruciei and Monhystera microphthalma. The assay measures survival and three independent indicators of the degree of inhibition of postembryonic development of the nematodes. Simple methods for the cultivation of both species and a protocol for long term cryogenic storage of D. bruciei are described.

The postembryonic development of D. bruciei is significantly inhibited at concentrations of 0.56 mg/mL potassium dichromate, 10^{-7} molar mercuric chloride, 3.2×10^{-7} molar 2-acetamidofluorine, 10^{-3} molar phenacetin and 10^{-5} molar 4-aminobiphenyl. Significant mortality of D. bruciei was detected at concentrations of 10 mg/l potassium dichromate, 10^{-6} molar mercuric chloride, 3.2×10^{-8} molar methyl mercury chloride, 3.2×10^{-3} molar phenacetin and 10^{-5} molar 4-aminobiphenyl.

Normal development of M. microphthalma was observed at concentrations of 10^{-6} molar mercuric chloride and 10^{-3} molar cesium chloride. Exposure of M. microphthalma to concentrations of 3.2×10^{-6} molar mercuric chloride, 3.2×10^{-3} molar cesium chloride or 10^{-5} molar selenium oxide causes high mortality.

A concentration of 10^{-6} molar selenium oxide or 0.1 mg/l sodium lauryl sulphate is inhibitory to the development of M. microphthalma.

The "nematode test" using D. bruciei detects toxicity at lower concentrations of 2 and at higher concentrations of 3 of the 5 chemicals tested than the nematode test using Panagrellus redivivus. P. redivivus is more sensitive to the chemicals tested than M. microphthalma. D. bruciei is a more convenient laboratory organism than M. microphthalma since it is easier to manipulate, grow in liquid culture medium and measure.

I

INTRODUCTION

Two conceptually similar approaches to detect and map the primary sites and sources of biotoxicity in an aquatic ecosystem have been proposed (Samoiloff and Wells, 1984). In the approach taken by Long (1983) the primary set of data upon which priorization of sites is based, is a survey of the chemicals in the field. In the approach taken by Samoiloff et al (1983a), the initial base for priorization of subsequent studies is based on data from bioassays conducted on environmental samples or extracts and chemical fractions of these samples. A bioassay used for this purpose should have the following characteristics:

1. the assay should be rapid, providing results for subsequent action within a limited period of time,
2. the bioassay should be cost-effective,
3. the bioassay should be capable of detecting severe toxic effects, such as lethality, as well as,
4. being capable of detecting subtle effects, such as mutagenesis,
5. the bioassay should be flexible in the type of the sample to be tested, being applicable to actual environmental samples, fractions of these samples, and to individual chemicals.

Such a bioassay should furthermore be using a test species which is:

6. representative of the taxon of which it is a member,
7. representative of the trophic level in the ecosystem examined, and

8. representative of the organisms present in the ecosystem under study.

These latter three points, however, are of secondary importance to the overall objective of detection of biological effects (biotoxicity). The use of a multicellular eucaryote test-organism, however, is preferable and the studied effects should be of some general biological significance.

On the other hand, a need has also been expressed for toxicity tests using ecologically important species of an ecosystem or representative species of taxa that occupy key positions in the different trophic levels of the specific ecosystem under study (Samoiloff and Wells, 1984). The general characteristics of such a bioassay should be similar to those discussed in 1-8 above, but more importance should be attached to (3) and (4), and, of course, the studied effects must be of some ecological significance.

Nematodes are the most abundant metazoans in littoral, estuarine, coastal, as well as oceanic sediments (Nicolas, 1975) and they usually comprise more than 90% of the metazoan fauna. A very significant portion of the energy flow of marine benthic systems passes through the nematodes (Heip et al, 1982). The systematics and ecology of free-living marine nematodes have recently been reviewed by Heip et al (1982). The use of nematodes in marine ecotoxicology has been reviewed by Samoiloff and Bogaert (1984).

A chronic, quantitative and cost-effective 96-hour toxicity test using a model organism for the study of genetics and development, the free-living nematode Panagrellus redivivus, has been developed and extensively applied to detect the toxicity of complex environmental samples (fish tissue

homogenates, mine tailings, crude oil, ureaformaldehyde insulation) as well as chemical fractions of river sediments and samples of indoor air. This "nematode test" quantitatively measures lethal, semilethal, sublethal as well as mutagenic or phenotoxic effects (Samoiloff, 1980; Samoiloff et al, 1980, 1983a, 1983b, in press). It has been used to determine the primary sites and sources of biotoxicity in a fresh water system through the generation of a map of the distribution of biotoxicity (Samoiloff et al. 1983a)

The post-embryonic development of P. redivivus consists of growth through three juvenile stages (L2, L3 and L4) and involves three moults. The growth rate decreases before each moult and juveniles continue their development only when appropriate stimuli have been received from the environment. A food stimulus required for the initiation of the L2 to L3 moult and a requirement for cholesterol at the L4 to adult moult have been identified (Samoiloff et al, 1980; Samoiloff, 1984). The synthesis of RNA, required to grow through a juvenile stage and to complete the moult to the next stage, occurs during or shortly after the previous moult (Boroditsky and Samoiloff, 1973). An order of magnitude more of gene activity is required to reach the adult stage than to reach the L3 or L4 stage. The successful completion of the L2 to L3 moult, L3 to L4 moult and L4 to adult moult requires the activation of respectively 75, 100 and 1000 X-linked genes per organism (Samoiloff 1980).

In the Panagrellus redivivus test, a population of 100 L2-juveniles, distributed 10 each in 10 autoanalyzer cups containing 0.5 mL of a minimal growth medium, are grown until 50% percent of the population is adult. The primary set of data from which toxicity is determined, consists of the

number of surviving nematodes as well their distribution over the four postembryonic stages in both the control and test populations (Samoiloff et al 1983a). The stage distribution is analyzed to provide evidence of three distinct toxicological effects:

1. Lethality (or semilethality), in which a significant proportion of the tested population dies, while the control population has no significant death.
2. Inhibition of physiological or developmental processes, in which the proportion of growth to each stage is inhibited relative to the controls. This effect indicates that exposure to the tested materials is inhibitory or debilitating to normal physiological processes.
3. Inhibition or damage to genetic mechanisms, which is manifested as the specific inhibition of the moult to the adult stage, relative to controls. Growth through the final moult requires extensive gene expression and this growth is highly sensitive to known mutagens.

The Panagrellus test appears to be slightly more sensitive in the detection of mutagens than the Salmonella typhimurium Ames test (Samoiloff et al, 1983a).

The object of this study was to develop a similar test protocol that can be used for the detection and monitoring of biotoxicity in a marine environment, but that also uses a species that is representative of an ecologically important group of the marine benthic microfauna. This required the selection of a marine nematode with a short life cycle, a postembryonic development that can be entrained to environmental stimuli in a manner similar to that observed for the postembryonic development of P.

redivivus, and that has similarly simple nutritional requirements. Methods for the cultivation of marine nematodes and species cultured were reviewed by Kinne (1977) and more recently by Heip et al (1982). In most instances marine nematodes that can be cultured with bacteria as food are grown in a substrate (agar) and the culture media are based on natural seawater, highly enriched with organic nutrients. Such a medium is unsuitable for toxicity testing since various interactions are possible between the toxicant and the culture medium (Samoiloff, 1980).

Diplolaimelloides bruciei and Monhystera microphthalma are two fairly large, abundant and cosmopolitan Monhysterid nematodes with a short life cycle. Culture methods have been developed and demographic parameters have been determined for these species by Warwick (1981) and Van Brussel (1979), respectively. Diplolaimelloides bruciei is an opportunistic species (Warwick, 1981) as is Panagrellus and might, therefore, entrain its development to its environment in a similar way as Panagrellus.

Work reported here was directed towards the determination of the utility of these two species as indicators of toxicity, as marine analogs of the Panagrellus bioassay.

II

MATERIALS AND METHODS

2.1 STOCK CULTURES

M. microphthalma was isolated in September 1982 from a polyhaline-marine pond in Belgium (the Spuikom in Ostend) and brought into culture using the methods described in Vranken et al (1981) and Geraert et al (1981). The meiobenthic organisms and detritus were extracted from the sediment by the method of Barnet (1968) and collected on a sieve (mesh width = 38 micron) after which the animals were removed and placed into petri-dishes containing 0.8% bacto-agar in seawater collected in the dievengat enriched with 1% Vlasblom medium and 15 g/l silica. After a few weeks nematodes, harpactoids and other organisms penetrate into the transparant agar. Gravid females of M. microphthalma were transferred to petri dishes containing the same culture medium.

Cultures from a strain of Diplolaimelloides bruciei isolated in England (Lynher Estuary) and a strain isolated from the Gulf of Mexico were obtained from Dr. R. Warwick and maintained as described in Warwick (1981). The culture medium, however, was constituted using artificial seawater (Dietrich and Kalle, 1963) instead of natural seawater.

Cultures of both species were also established in a medium developed for Panagrellus redivivus (Samoiloff et al, 1980) adapted for marine conditions by replacing the M9-saline by artificial seawater. The concentration of cholesterol in the agar and the feeding solution was doubled. M.

microphthalma and D. bruciei were maintained in petri-dishes on a 2% agar containing 10 mg cholesterol and 25 g artificial seasalt (Instant Ocean) per liter distilled water (CS-agar). The animals were fed 1 to 3 drops per week of YCS-solution (Yeast-Cholesterol-Saline) containing 50 mg autoclaved dried baker's yeast, 10 mg cholesterol and 25 g artificial seasalt (Instant Ocean) per liter of distilled water.

To obtain standardized cultures it is important not to overfeed the animals and to keep them confined to a capillary layer of water at the air-agar interface, wherein the animals can crawl slowly, but are unable to swim.

More or less inbred strains of both species were constructed by approximately 10 generations of sib-sib matings.

Diplolaimelloides bruciei can be stored in the freezer for at least 9 months using the following procedure:

1. One mL of a 3:1 mixture of YCS and glycerine is pipetted into an exponentially growing stock culture containing several hundred animals.
2. The suspended animals are distributed in 2 autoanalyzer cups.
3. The cups are placed immediately in a styrofoam box, which is placed in a freezer at -80 degrees centigrade.
4. To re-establish stock cultures one cup is removed from the freezer and placed at room temperature. As soon as the contents of the cup thaws, as much of the freezing solution as possible is pipetted off using a micropipette and the animals are resuspended in YCS and subsequently transferred to a petri dish (12 cm diameter) with CS-agar, that is flooded with 2-3 mL of YCS. Two to three hours

later some of the recovered animals are transferred to new petridishes to establish new stock-cultures.

2.2 DETERMINATION OF THE BODY LENGTHS OF THE STAGES

The body lengths at which juveniles of Panagrellus redivivus moult vary with the culture conditions under which the animals are grown (Samoiloff, personal communication). Therefore, populations of the two marine nematode species studied were measured to determine the body length at which the juveniles moult, under the culture conditions employed in this study. Animals were mounted on slides and measured as described in the test-protocol. It is to be noted that the animals shrink somewhat in the mounting procedure.

Samoiloff (1984) demonstrated that P. redivivus requires a food stimulus to complete the L2 to L3 moult and that cholesterol is required to complete the L4 to adult moult. M. microphthalma and D. bruciei have similar requirements.

To determine the maximum body size of L2-juveniles, freshly hatched juveniles were placed 10 by 10 in autoanalyzer cups with 0.5 mL YCS, from which the yeast was omitted, incubated for 5 days and measured. To determine the maximum body size of L4-juveniles, freshly hatched juveniles were placed 10 by 10 in autoanalyzer cups with 0.5 mL YCS from which the cholesterol was omitted, incubated for 9 days and subsequently measured.

The growth rate of P. redivivus decreases at the time of each moult and growth is resumed immediately after the moult. Caenorhabditis and Panagrellus are in a state of lethargus shortly before and during the moult (Samoiloff and Boroditsky 1973, Sternberg and Horvitz, 1982). They thus

spend a longer time at the body lengths at which they moult than at any specific intermoult body length.

Freshly hatched juveniles were placed 10 by 10 in autoanalyzer cups and incubated at 28 degrees centigrade. At 24 hour intervals five cups were removed. The nematodes were mounted on slides, measured as described below and the frequency distribution determined for different size classes. The body length at which L2-, L3- and L4-juveniles moult was determined, by determining the length classes at which the juveniles accumulate. The length measurements of the nematodes, grown in toxicants, that were clearly blocked at a moult were also used to determine the maximum body length at which the nematodes moult.

2.3 SYNCHRONIZATION OF L2 JUVENILES

Synchronized L2-juveniles, for growth experiments or toxicity tests are obtained by transferring gravid females to a fresh culture medium, using a needle, (without adding YCS) and allowing them to drop eggs for 48 hours at 28 degrees Centigrade. Starved L2 juveniles of similar size are collected 48 hours later with a micropipette after flooding the agar with YCS-solution from which the yeast was omitted. If, for an experiment, more juveniles are required than can be produced in one petri dish, the juveniles produced in several petri dishes are pooled to eliminate differences between petri dishes.

2.4 PROTOCOL OF THE TOXICITY TEST

The test protocol is a modification of the test protocol developed for P. redivivus (Samoiloff et al, 1980).

Synchronized L2 juveniles are transferred 10 by 10 to autoanalyzer cups with 0.3 or 0.5 mL of toxicant dissolved in YCS containing only 15 mg yeast per liter. Hydrophobic toxicants are tested in YCS with up to 5 mL/l acetone. The sealed autoanalyzer cups are incubated until 50% of the animals in the control series grow to the adult stage.

The surviving animals of each series of ten cups are transferred to a small drop of liquid on a slide, heat-killed and dried on a slidewarmer at approximately 45 degrees centigrade and mounted in lacto-phenol cotton blue. The nematodes on the slide are photographed using a microfilm viewer equipped with a photocopier and measured using a Houston Hipad Digitizer interfaced to an Apple computer. The lengths of the nematodes of each sample are stored on floppy disks for further analysis.

2.5 DATA TREATMENT AND INTERPRETATION OF THE RESULTS

The total number of L2, L3, L4 and adult animals are determined for each sample, using the length of the animals as criterion and the frequency of completion of the L2-L3 molt (p1), L3-L4 molt (p2) and L4-adult molt (p3) values are calculated (Samoiloff, 1980).

The development of the nematodes in the experimental sample (usually YCS to which toxicant was added with or without carrier) is compared to the development of the nematodes in the control sample (usually YCS or YCS with 5 mL/l acetone), using the following procedure:

1. The percent survival in the control sample is compared to the percent survival in the experimental sample using a chi-square test to determine whether significant mortality occured in the experimental sample as compared to the control sample.
2. The stage distribution of the animals grown in the experimental sample is compared to the stage distribution of the animals grown in the control sample using a 2 x 4 contingency analyses (chi-square test) (Samoiloff et al, 1980).
3. The relative ratios of p1, p2, and p3 (experimental/control) are calculated (P1, P2 and P3).

III

RESULTS

3.1 GROWTH CHARACTERISTICS OF MARINE NEMATODES ON CS-AGAR

On CS-agar at 28 degrees centigrade the embryonic development of *M. microphthalma* requires 1.2 to 2 days and the postembryonic development requires 5.5 to 10 days. The average fecundity under these conditions is 7.2 eggs per female per day. Under the same culture conditions the embryonic development of *D. bruciei* requires 1.5 to 2 days while the postembryonic development requires 5 to 11 days. The fecundity is 2 - 11 eggs per female per day (average 6.7). A female of *D. bruciei* produces up to 132 eggs throughout her life. Up to 27 fertile sperm are transferred per mating.

In stock cultures the animals are kept confined in a capillary layer of water on the agar, since the efficiency of copulation and egg laying is reduced in free-swimming *M. microphthalma* and *D. bruciei* as well as in free-swimming *P. redivivus*.

3.2 THE LENGTHS OF THE POSTEMBRYONIC STAGES

The maximum body lengths of the L2-, L3- and L4-juveniles of *D. bruciei* were 370 , 470 and 700 microns respectively. Most L2-, L3- and L4-juveniles of *D. bruciei* appeared to moult at 310, 430 and 675 microns, respectively.

The maximum body lengths of L2-, L3- and L4-juveniles of M. microphthalma were 360, 475 and 650 microns, respectively.

3.3 POST-EMBRYONIC DEVELOPMENT IN YCS

Synchronized juveniles of D. bruciei and M. microphtalma were placed 10 by 10 in autoanalyzer cups with YCS and incubated at 25 degrees centigrade. At 24 hour intervals 5 cups were removed, the nematodes were mounted on slides, measured and the number of L2-, L3-, L4- juveniles and adults was determined. The results are shown in table I. The pattern of development in YCS of the two species is similar. Most juveniles of D. bruciei (Lynher strain), D. bruciei (Gulf of Mexico strain) and M. microphthalma have completed the L2 to L3 moult by days 4, 6 and 5, respectively. When L4 animals appear in the population, a minimum 60% of the nematodes have completed the L2 to L3 moult. More than 95% of the juveniles of D. bruciei (Lynher strain), D. bruciei (Gulf of Mexico strain) and M. microphthalma have completed the L3 to L4 moult by days 6, 7 and 6, respectively. When adults appear in the population, more than 95% of the nematodes have reached the L3-stage and minimum 85% of the nematodes have reached the L4-stage.

L2-juveniles of P. redivivus were placed 10 by 10 in autoanalyzer cups with 0.5 mL YCS with a salinity of 10, 20, 30 and 40%. No growth was observed after 8 days. All life stages of P. redivivus, however, survive in seawater of 30% salinity for 9 days.

D. bruciei has a larger body width than M. micropthalma. D. bruciei is, therefore, more easy to manipulate, stretches better when heat-killed on a slide, and is easier to observe in vivo or mounted on a slide. M.

micropthalma has a characteristic long slender tail, while *D. bruciei* has a blunt tail. Juvenile stages of *D. bruciei* have more distinct size classes than juvenile stages of *M. microphthalma*. The length and juvenile stage of *D. bruciei* can, therefore, be determined more conveniently and precisely. In liquid culture medium *M. microphthalma* swim hyperactively unless they can surround themselves with a small pellet of detritus.

D. bruciei appears less stressed in liquid culture medium and has a lesser tendency to crawl into pellets.

The concentration of dried yeast in the growth medium YCS was reduced to 15 microgram per mL (7.5 microgram per autoanalyzer cup), to reduce the formation of pellets by the nematodes. Juveniles of *D. bruciei* can be readily blocked at the L2 stage by omitting yeast from the YCS and at the L4 stage by omitting cholesterol from the growth medium (Table II). The juveniles are blocked at the body lengths corresponding to the maximum sizes of the different juvenile stages and readily resume growth upon feeding. The life strategies of *D. bruciei* and *P. redivivus* are in this respect similar. Starved juveniles of *M. microphthalma* resume growth less readily.

The results of two experiments wherein *D. bruciei* (Lynher) was grown at different densities in autoanalyzer cups with 0.3 or 0.5 mL YCS are reported in Table III. No significant difference in growth was observed between cups containing 10 animals in 0.3 and 0.5 mL YCS and cups containing 5 animals in 0.3 mL and cups containing 10 animals in 0.3 and 0.5 mL YCS and cups containing 5 animals in 0.3 or 0.5 mL YCS. The nematodes grew significantly better in cups with 2 animals and 0.3 mL YCS than in cups with 5 and 10 animals and 0.3 mL YCS. In both experiments the

nematodes grew significantly better in cups with 10 animals and 0.5 mL YCS than in cups with 20 animals and 0.5 mL YCS. In both experiments the animals grew significantly better in cups with 10 animals in 0.5 mL YCS than in cups with 10 animals and 0.3 mL YCS.

3.4 REPRODUCIBILITY OF THE TEST

The stage distribution of the nematodes grown in five groups of 10 cups containing each 10 synchronized L2-juveniles 0.5 mL YCS, were compared to each other as described above. The results for *D. bruciei* are reported in Tables IV and V, and the results for *M. microphthalma* are reported in Table VI. Due to the variation in observed mortalities I consider a tested sample as "causing significant mortality", only when there is at least a 25% difference in survival between the control and experimental populations. An important reason for the large differences in observed mortality in these controls is that some of the nematodes can not be observed or separated from the "pellets" of detritus they collect around themselves (possibly with secretions from their caudal glands). The stage distribution of the nematodes grown in the experimental will be considered significantly different from the stage distribution of the animals grown in the control only when a difference is detected at the 0.001 level.

3.5 EXPOSURE - RESPONSE STUDIES WITH VARIOUS TOXICANTS

The test-protocol was tested by using reference chemicals and heavy-metal compounds as well as known mutagens or carcinogens. The effect of potassium dichromate on *D. bruciei* is reported in Table VII. Significant mortality of *D. bruciei* is observed at a concentration of 10 mg/l potassium dichromate.

The development of D. bruciei is significantly inhibited at concentrations of 0.56 to 1 mg/l. The third and fourth moult are completely inhibited (P2 = 0, P3 = 0) at a lower concentration of potassium dichromate than the second moult. The second moult however is more than 70% inhibited at a concentration of 1.8 mg/l potassium dichromate. When the P1 value is low, the calculation of the P2 and P3 is based on a small number of nematodes and therefore more susceptible to variation.

The results of the three tests with potassium dichromate are comparable between the two tested species. A concentration of 5 mg/l potassium dichromate significantly inhibits the development of M. microphtalma. At a concentration of 10 mg/l all nematodes are arrested at the L2 stage. At concentrations of 20 and 40 mg/l the survival is respectively 38% and 36% (Table XI).

Mercuric (II) chloride significantly inhibits the L4 to adult moult of D. bruciei (Lynher strain) at a concentration of 10^{-7} molar. Both the third and fourth moult are inhibited at a concentration of 10^{-6} molar. A concentration of 3.2×10^{-6} molar was 100% lethal in two experiments. In a third experiment 35% survival, but no growth was observed at this concentration. A distinct but non-significant inhibition of the L4 to adult moult of the Mexican strain of D. bruciei was observed at 10^{-7} and 10^{-6} molar mercuric (II) chloride. No survival was observed at a concentration of 3.2×10^{-6} molar mercuric chloride (Table VIII.). At concentrations of 10^{-7} and 10^{-6} molar mercuric chloride, M. microphthalma grows as well as the control. At a concentration of 3.2×10^{-6} molar no survival was observed (Table XI). Methyl mercury chloride is not inhibitory to the development of D. bruciei at a concentration of 10^{-8}

molar, but it is lethal to 98% of the nematode population at a concentration of 3.2×10^{-8} molar (Table X).

At a concentration of 3.2×10^{-3} molar, cesium chloride strongly inhibits the last three moults of M. microphthalma and is lethal to 65% of the nematode population. No effect is observed at a concentration of 10^{-3} molar. A concentration of 3.2×10^{-2} molar is 100% lethal to the nematodes (Table XI).

Exposure to 10^{-4} molar selenium oxide is 100% lethal to M. microphthalma. At 10^{-6}, and 10^{-5} molar, the L4-adult moult is strongly inhibited and the L3-L4 moult slightly inhibited (Table XI).

Exposure to 0.1 mg/l sodium lauryl sulphate inhibits the development of M. microphthalma. At a concentration of 20 mg/l, 70% mortality and no growth of the surviving nematodes is observed (Table XI.).

The known carcinogen, 2-acetamidofluorine, significantly inhibits development of D. bruciei (Lynher strain) at a concentration of 3.2×10^{-7} molar (Table IX). At concentrations above 10^{-5} molar, a sharp increase in the inhibition of development is observed (Table IX).

At a concentration of 10^{-3} molar, the putative carcinogen phenacetin inhibits the development of D. bruciei (Lynher). A concentration of 3.2×10^{-3} molar is 100% lethal to D. bruciei. The L4 to adult moult of D. bruciei is slightly inhibited (not significant the .001 level) at a concentration of 10^{-4} molar phenacetin. (Table X).

The last three moults of D. bruciei (Lynher strain) are inhibited at a concentration of 10^{-5} molar 4-aminobiphenyl. A concentration of 10^{-6} molar 4-aminobiphenyl does not alter the pattern of post-embryonic development of D. bruciei. At a concentration of 10^{-4} molar 95% mortality and no growth were recorded.

TABLE I. Postembryonic development of *Diplolaimelloides bruciei* and *Monhystera microphthalma* in YCS.

	D. bruciei (L.)			*D. bruciei* (M.)			*M. microphthalma*		
Day	p1	p2	p3	p1	p2	p3	p1	p2	p3
1	0	-	-	0	-	-	0	-	-
2	0	-	-	0	-	-	0	-	-
3	0.72	0.08	0	0.62	0.1	0	0.04	0	-
4	1.00	0.82	0	0.76	0.39	0	0.76	0.05	0
5	1.00	0.90	0.04	0.96	0.41	0	1.00	0.88	0
6	1.00	0.98	0.25	1.00	0.85	0.05	1.00	0.98	0.19
7	0.94	0.94	0.44	0.96	0.98	0.65	0.97	0.95	0.28
8	1.00	0.97	0.64	0.97	1.00	0.70	1.00	0.93	0.66
9	1.00	0.98	0.78						
10	1.00	0.98	0.95						

Note :

The values p1, p2, p3 represent the frequency of successfull development through the L2 to L3, L3 to L4 and L4 to adult moults respectively (Samoiloff, 1980) .

TABLE II. Post-embryonic development of *Diplolaimelloides bruciei* in liquid culture medium without cholesterol (YS)

A. British strain

Sample	Survivors	Measured	L2	L3	L4	Ad.	p1	p2	p3
YCS	98	98	0	3	34	61	1	0.97	0.64
YS	96	96	0	11	85	0	1	0.89	0

Contr.	Exper.	%-surv.	Sign. diff.	P1	P2	P3	Chi-square value	Sign. diff.
YCS	YS	98	no	1	0.91	0	90.6	yes

B. Mexican strain

Sample	Survivors	Measured	L2	L3	L4	Ad.	p1	p2	p3
YCS	100	100	2	8	47	43	0.98	0.92	0.48
YS	95	95	1	22	68	4	0.99	0.77	0.06

Contr.	Exper.	%-surv.	Sign. diff.	P1	P2	P3	Chi-square value	Sign. diff.
YCS	YS	95	no	1.01	0.83	0.12	44.6	yes

(Continued on next page)

(Table II continued)

Note :

The values p1, p2, p3 represent the frequency of successfull development through the L2 to L3, L3 to L4 and L4 to adult moults respectively (Samoiloff, 1980) ;

"Survivors" = nr. of surviving nematodes in the sample;

"Measured" = nr. of nematodes of the sample that were measured;

"L2", "L3", "L4" and "Ad." = nr. of L2-, L3-, L4-juveniles and adults in the sample;

P1 = p1 of the experimental divided by p1 of the control;

P2 = p2 of the experimental divided by p2 of the control;

P3 = p3 of the experimental divided by p3 of the control.

TABLE III. Effect of the quantity of YCS and the number of animals per cup on the post-embryonic development of Diplolaimelloides brucei.

Volume per cup (mL)	Animals per cup	%-survival	p1	p2	p3
0.3	10	84	0.95	0.79	0.40
0.3	5	90	0.89	0.93	0.41
0.3	2	100	1.00	1.00	1.00
0.3	20	72	0.94	0.93	0.34
0.5	10	89	1.00	0.97	0.92
0.5	20	89	0.91	0.89	0.41

Control (mL-an.)	Experimental (mL-an.)	%-survival (vs. contr.)	Chi-square value	Significant difference
0.3 - 10	0.3 - 5	107	1.59	no
0.3 - 10	0.3 - 2	119	17.39	no
0.3 - 10	0.3 - 20	86	4.20	no
0.3 - 10	0.5 - 10	106	1.07	no
0.3 - 20	0.5 - 20	124	9.21	no
0.5 - 10	0.5 - 20	100	0	no

(Continued on next page)

(Table III continued)

Control (mL-an.)	Experimental (mL-an.)	P1	P2	P3	Chi-square value	Significant difference
0.3 - 10	0.3 - 5	0.93	1.16	1.01	9.01	no
0.3 - 10	0.3 - 2	1.05	1.26	2.48	106.54	yes
0.3 - 10	0.3 - 20	0.99	1.17	0.85	7.55	no
0.3 - 10	0.5 - 10	1.05	1.22	2.28	70.94	yes
0.3 - 20	0.5 - 20	0.96	0.96	1.20	2.50	no
0.5 - 10	0.5 - 20	0.91	0.92	0.46	66.34	yes

Volume per cup (mL)	Animals per cup	%-survival	p1	p2	p3
0.5	10	89	1.00	0.93	0.54
0.5	5	92	0.97	0.98	0.57
0.5	20	93	0.90	0.86	0.27
0.3	10	98	0.96	0.90	0.27

(Continued on next page)

(Table III continued)

Control (mL-an.)	Experimental (mL-an.)	%-survival (vs. contr.)	Chi-square value	Significant difference
0.5 - 10	0.5 - 5	103	0.52	no
0.5 - 10	0.5 - 20	104	0.98	no
0.5 - 10	0.3 - 10	110	6.66	no

Control (mL-an.)	Experimental (mL-an.)	P1	P2	P3	Chi-square value	Significant difference
0.5 - 10	0.5 - 5	0.97	1.05	1.06	5.88	no
0.5 - 10	0.5 - 20	0.90	0.92	0.50	25.71	yes
0.5 - 10	0.3 - 10	0.96	0.92	0.49	18.51	yes

TABLE IV. Reproducibility of the test-protocol (D. bruciei)

10 animals and 0.5 ml YCS per cup.

Sample	Survivors	Measured	L2	L3	L4	AD.	p1	p2	p3
1	90	82	3	5	30	44	0.96	0.94	0.59
2	76	72	6	3	21	42	0.92	0.95	0.67
3	88	86	2	3	41	40	0.98	0.96	0.49
4	94	88	0	7	34	47	1.00	0.92	0.55
5	92	85	0	1	38	46	1.00	0.99	0.55

Control	Experimental	%-Survival	Chi-square value	Level of significance
1	2	84	6.95	0.01
1	3	98	0.20	n.s.
1	4	104	1.09	n.s.
1	5	102	0.24	n.s.
2	3	116	4.88	0.05
2	4	124	12.71	0.001
2	5	121	9.52	0.01
3	4	107	2.20	n.s.
3	5	105	0.89	n.s.
4	5	98	0.31	n.s.

(Continued on next page)

(Table IV continued)

Contr.	Exper.	P1	P2	P3	Chi-square value	Level of significance
1	2	1.07	1.01	0.74	9.24	0.05
1	3	1.09	0.96	0.87	11.06	0.05
1	4	1.09	1.04	0.82	13.43	0.01
2	3	1.07	1.01	0.74	9.24	0.05
2	4	1.09	0.96	0.87	11.06	0.05
2	5	1.09	1.04	0.82	13.46	0.01
3	4	1.02	0.95	1.17	5.49	n.s.
3	5	1.02	1.02	1.11	4.14	n.s.
4	5	1.00	1.07	0.94	5.48	n.s.

TABLE V. Reproducibility of the test protocol (*D. bruciei*)

10 animals and 0.5 mL YCS per cup.

Sample	Survivors	Measured	L2	L3	L4	AD.	p1	p2	p3
1	98	86	0	2	31	53	1.00	0.98	0.63
2	94	91	0	6	38	47	1.00	0.93	0.55
3	81	78	1	7	24	46	0.99	0.91	0.66
4	83	80	2	0	25	53	0.98	1.00	0.68
5	100	98	3	3	35	57	0.97	0.97	0.62

Control	Experimental	%-Survival	Chi-square value	Level of significance
1	2	96	2.08	n.s.
1	3	83	15.38	0.001
1	4	85	13.09	0.001
1	5	102	2.02	n.s.
2	3	86	7.73	0.01
2	4	88	5.94	0.05
2	5	106	6.19	0.05
3	4	102	0.14	n.s.
3	5	123	20.99	0.001
4	5	120	18.55	0.001

(Continued on next page)

(Table V continued)

Contr.	Exper.	P1	P2	P3	Chi-square value	Level of significance
1	2	1.00	0.96	0.88	3.34	n.s.
1	3	0.99	0.93	1.04	5.67	n.s.
1	4	0.98	1.02	1.08	5.33	n.s.
1	5	0.97	0.99	0.98	3.26	n.s.
2	3	0.99	0.97	1.19	3.80	n.s.
2	4	0.98	0.97	1.23	12.41	0.01
2	5	0.97	1.04	1.12	5.21	n.s.
3	4	0.99	1.04	1.03	9.79	0.05
3	5	0.98	1.07	0.94	4.01	n.s.
4	5	0.99	0.97	0.94	3.94	n.s.

TABLE VI. Reproducibility of the test protocol (*M. microphthalma*)

10 animals and 0.5 ml YCS per cup.

Sample	Survivors	Measured	L2	L3	L4	AD.	p1	p2	p3
1	83	82	0	9	30	43	1.00	0.89	0.59
2	94	93	3	4	46	40	0.97	0.96	0.47
3	84	79	4	5	25	45	0.95	0.93	0.64
4	87	82	3	8	34	37	0.96	0.90	0.52
5	92	89	0	0	42	47	1.00	1.00	0.53

Control	Experimental	%-Survival	Chi-square value	Level of significance
1	2	113	5.94	0.05
1	3	101	0.04	n.s.
1	4	105	0.63	n.s.
1	5	111	3.70	0.1
2	3	89	5.11	0.05
2	4	93	2.85	0.1
2	5	98	0.31	n.s.
3	4	104	0.36	n.s.
3	5	110	3.03	0.1
4	5	106	1.33	n.s.

(Continued on next page)

(Table VI continued)

Contr.	Exper.	P1	P2	P3	Chi-square value	Level of significance
1	2	0.97	1.07	0.79	9.00	0.05
1	3	0.95	1.05	1.09	6.86	0.1
1	4	0.96	1.01	0.88	4.58	n.s.
1	5	1.00	1.12	0.90	12.32	0.01
2	3	0.98	0.98	1.38	6.65	0.1
2	4	1.00	0.94	1.12	2.90	n.s.
2	5	1.03	1.05	1.14	8.58	0.05
3	4	1.01	0.96	0.81	3.65	n.s
3	5	1.05	1.07	0.82	14.61	0.01
4	5	1.04	1.11	1.01	14.39	0.01

TABLE VII. Effect of potassium dichromate on the development of *D. bruciei* (Lynher).

Concentr. (mg/l)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
1.0	88	4.88	no	0.55	0.81	0.93	66.7	yes
1.8	89	4.15	no	0.29	0.49	0.61	137.4	yes
3.2	91	2.83	no	0.21	0.60	0.50	143.21	yes
5.6	75	16.23	no	0.03	0	-	186.34	yes
10.0	40	65.26	yes	0	-	-	191.4	yes
13.5	5	148.16	yes	0	-	-	191.4	yes
18.0	0	-	yes	-	-	-	-	-
20.0	0	-	yes	-	-	-	-	-

Concentr. (mg/l)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
0.56	86	15.05	no	0.93	0.91	1.19	13.61	no
1.0	98	2.02	no	0.55	0.80	0.83	71.91	yes
1.8	88	12.77	no	0.19	0.91	0.46	138.45	yes
3.2	80	22.22	no	0.18	0.81	0.42	145.36	yes
5.6	87	13.90	no	0.21	0.75	0	144.53	yes
10.0	62	46.91	yes	0.05	0	-	192.41	yes
13.5	24	122.58	yes	0	-	-	200	yes
18.0	17	141.88	yes	0	-	-	200	yes

(Continued on next page)

(Table VII continued)

Concentr. (mg/l)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
0.56	81	17.74	no	0.76	0.83	0.76	38.10	yes
1.0	92	5.67	no	0.64	0.88	0.67	51.29	yes
1.8	94	3.79	no	0.26	0.84	0	126.71	yes
3.2	93	4.71	no	0.20	0.51	0.51	149.98	yes
5.6	78	21.4	no	0.11	0.13	0	180.20	yes
10.0	68	33.28	yes	0.06	0	-	185.93	yes
13.5	18	131.36	yes	0.11	0	-	185.12	yes
18.0	11	152.62	yes	0	-	-	192.00	yes

TABLE VIII. Effect of mercuric (II) chloride on the development of *D. bruciei* (Lynher).

English strain

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
1.0×10^{-7}	97	3.05	no	1.00	0.94	0.78	11.8	no
1.0×10^{-6}	90	10.53	no	0.91	0.79	0.11	106.3	yes
3.2×10^{-6}	0	-	yes	-	-	-	-	-
3.2×10^{-5}	0	-	yes	-	-	-	-	-

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
3.2×10^{-8}	104	4.08	no	1.00	1.02	0.96	0.9	no
10^{-7}	100	0	no	1.00	0.98	0.62	6.7	yes
3.2×10^{-7}	85	10.01	no	0.97	0.86	0.60	17.81	yes
10^{-6}	93	3.53	no	0.97	0.69	0.08	64.59	yes
3.2×10^{-6}	35	84.48	yes	0	-	-	200	yes

(Continued on next page)

(Table VIII continued)

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
3.2×10^{-8}	98	0.13	no	1.02	0.97	1.2	3.6	no
10^{-7}	98	0.13	no	0.91	0.87	0.57	20.7	yes
3.2×10^{-7}	54	29.31	yes	0.38	0.62	0	110.7	yes
10^{-6}	26	72.03	yes	0.30	0.52	0	124.2	yes
3.2×10^{-6}	0	-	yes	-	-	-	-	-

Mexican strain

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
1.0×10^{-7}	100	0	no	0.98	0.95	0.77	4.3	no
1.0×10^{-6}	100	0	no	0.96	0.87	0.78	12.3	no
3.2×10^{-6}	0	-	yes	-	-	-	-	-
3.2×10^{-5}	0	-	yes	-	-	-	-	-

TABLE IX. Effect of 2-acetamidofluorine on the development of *Diplolaimelloides bruciei*.

British strain

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
10^{-8}	87	9.83	no	0.97	1.03	0.97	1.7	no
10^{-7}	92	4.92	no	0.91	1.02	0.84	8.5	no
10^{-6}	82	14.20	no	0.94	0.93	0.73	11.0	no
10^{-5}	67	33.27	yes	0.74	0.77	0.14	78.0	yes
10^{-4}	9	155.44	yes	0.46	0	-	157.6	yes

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
10^{-8}	87	2.54	no	0.98	0.99	0.85	1.2	no
3.2×10^{-8}	97	0.11	no	1.01	1.01	0.76	2.4	no
10^{-7}	103	0.12	no	0.92	0.98	1.13	4.9	no
3.2×10^{-7}	53	28.41	yes	0.74	0.78	0.32	49.1	yes
10^{-6}	50	31.33	yes	0.77	0.88	0.77	26.0	yes
3.2×10^{-6}	58	23.00	yes	0.79	0.84	0.45	34.2	yes
10^{-5}	47	34.39	yes	0.80	0.71	0.25	51.0	yes
3.2×10^{-5}	14	90.88	yes	0	-	-	-	yes
10^{-4}	22	74.61	yes	0	-	-	-	yes

(Continued on next page)

(Table IX continued)

Mexican strain

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
10^{-8}	108	4.71	no	1.00	1.00	1.06	0.2	no
10^{-7}	100	0	no	0.99	1.01	0.18	2.4	no
10^{-6}	101	0.06	no	0.99	0.98	0.84	2.4	no
10^{-5}	107	3.19	no	0.98	0.95	0.74	6.4	no
10^{-4}	62	31.45	yes	0.67	0.34	0	123.6	yes
10^{-3}	0	-	yes	-	-	-	-	-

TABLE X. Effects of methyl mercury chloride, phenacetin and 4-aminobiphenyl on the development of D. bruciei (Lynher).

Methyl mercury chloride

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
3.2×10^{-10}	89	4.73	no	1.00	1.01	0.92	1.5	no
1.0×10^{-9}	108	7.25	no	1.00	1.00	0.93	1.2	no
3.2×10^{-9}	87	6.37	no	1.00	0.92	0.93	2.7	no
1.0×10^{-8}	108	7.25	no	0.99	1.02	0.94	3.1	no
3.2×10^{-8}	2	166.04	yes	0	-	-	200	yes

Phenacetin

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
1.0×10^{-4}	95	5.13	no	1.00	0.97	0.57	9.8	no
3.2×10^{-4}	90	10.53	no	0.96	0.97	0.77	4.9	no
1.0×10^{-3}	93	7.25	no	0.99	0.81	0.06	58.3	yes

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(Table X continued)

4-aminobiphenyl

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
10^{-8}	90	1.09	no	1.02	0.99	1.09	1.5	no
10^{-7}	106	6.19	no	1.03	1.03	0.92	2.7	no
10^{-6}	91	3.56	no	0.93	1.00	1.13	4.9	no
10^{-5}	48	56.63	yes	0.63	0.55	0.47	78.5	yes
10^{-4}	5	158.44	yes	0	-	-	182.0	yes

TABLE XI. Effects of mercuric chloride, cesium chloride, selenium oxide, potassium dichromate and sodium lauryl sulphate on Monhystera microphthalma

Mercuric chloride

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
1.0×10^{-7}	100	0	no	0.98	0.95	0.99	5.8	no
1.0×10^{-6}	100	0	no	0.99	1.00	0.93	1.3	no
3.2×10^{-6}	0	-	yes	-	-	-	-	-
1.0×10^{-5}	0	-	yes	-	-	-	-	-

Cesium chloride

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
1.0×10^{-3}	89	5.56	no	1.00	1.00	0.90	1.0	no
3.2×10^{-3}	35	83.42	yes	0.61	0.26	0	145.1	yes
1.0×10^{-2}	21	115.09	yes	0	-	-	-	yes
3.2×10^{-2}	0	-	yes	-	-	-	-	-

(Continued on next page)

(Table XI continued)

Selenium oxide

Concentr. (molar)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
10^{-8}	98	0.69	no	0.99	0.98	0.98	0.5	no
10^{-7}	96	2.08	no	1.00	0.96	1.02	1.8	no
10^{-6}	78	21.40	no	1.02	0.84	0.32	66.0	yes
10^{-5}	53	56.43	yes	1.03	0.85	0.17	91.3	yes
10^{-4}	0	-	yes	-	-	-	-	-

Potassium dichromate

Concentr. (mg/l)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
5	106	6.19	no	0.64	0.79	1.17	42.3	yes
10	77	17.50	no	0	-	-	176.1	yes
20	38	73.93	yes	0	-	-	176.1	yes
40	36	78.13	yes	0	-	-	176.1	yes

(Continued on next page)

(Table XI continued)

Sodium lauryl sulphate

Concentr. (mg/l)	%-surv.	Chi-sq. value	Sign. diff.	P1	P2	P3	Chi-sq. value	Sign. diff.
0.1	80	15.46	no	0.77	0.78	1.19	42.29	yes
1	82	13.21	no	0.86	0.85	0.79	20.83	yes
5	80	15.46	no	0.84	0.76	0.24	62.71	yes
10	58	43.86	yes	1.03	0.88	0.30	46.2	yes
20	30		yes	0	-	-	-	yes
40	0	-	yes	-	-	-	-	yes

TABLE XII. Comparison of the sensitivity of the D. bruciei, M. microphthalma and P. redivivus nematode test for the detection of the toxicity of some of the tested chemicals.

MERCURIC CHLORIDE

	D. bruciei	M. microphthalma	P. redivivus
10^{-7} molar	P3 reduced	no effect	no effect
10^{-6} molar	mortality P1, P2, P3 reduced	no effect	P3 reduced
3.2×10^{-6} molar	mortality no growth	100% mortality	not tested
10^{-5} molar	100% mortality	100% mortality	100% mortality

METHYL MERCURY CHLORIDE

	D. bruciei	P. redivivus
10^{-8} molar	no effect	P2 and P3 reduced
3.2×10^{-8} molar	100% mortality	not tested
10^{-7} molar	"	P1, P2 and P3 reduced
10^{-6} molar	"	semi-lethality
10^{-5} molar	"	100% mortality

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(Table XII continued)

2-ACETAMIDOFLUORENE

	D. bruciei	P.redivivus
10^{-5} molar	Lethality P1, P2 and P3 reduced	Stimulatory effect
10^{-4} molar	91% lethality	no effect
10^{-3} molar	100% lethality	P2 and P3 reduced

PHENACETIN

	D. bruciei	P. redivivus
10^{-8} molar	no effect	P2 and P3 reduced
10^{-4} molar	no effect	P2 and P3 reduced
10^{-3} molar	100% lethality	P2 and P3 reduced

(Continued on next page)

(Table XII continued)

4-AMINOBIPHENYL

	D. bruciei	*P. redivivus*
10^{-8} molar	no effect	P1 and P3 reduced
10^{-6} molar	no effect	P1, P2 and P3 reduced
10^{-5} molar	P1, P2 and P3 reduced	P1, P2 and P3 reduced
10^{-4} molar	95% mortality	100% mortality

SELENIUM OXIDE

	M. microphthalma	*P. redivivus*
10^{-8} molar	no effect	P2 and P3 reduced
10^{-7} molar	no effect	P2 and P3 reduced
10^{-6} molar	P3 reduced	P2 and P3 reduced
10^{-5} molar	47% mortality	P2 and P3 reduced
10^{-4} molar	100% mortality	significant mortality P1, P2 and P3 reduced

(Continued on next page)

(Table XII continued)

CESIUM CHLORIDE

	M. microphthalma	*P. redivivus*
10^{-5} molar	no effect	P1 and P3 reduced
10^{-3} molar	no effect	P3 reduced
3.2×10^{-3} molar	65% mortality	"
10^{-2} molar	79% mortality	"
3.2×10^{-2} molar	100% mortality	"

Note :

The results from the toxicity tests with *P. redivivus* were obtained from Samoiloff *et al.* (1980).

IV

DISCUSSION

Culture media for M. microphthalma and D. bruciei were developed that are chemically less complex and less rich in organic compounds than the available culture media for M. microphthalma and D. bruciei, the media developed are suitable for toxicological studies. The generation time on YCS-agar and the postembryonic growth rate in YCS are in accordance with the development times recorded for M. microphthalma (Van Brussel, 1980) and D. bruciei (Warwick, 1981).

The toxicants tested in this study were selected on the basis of their known toxicological properties and because of their record as reference toxicants in ecotoxicology. Potassium dichromate and the anionic detergent sodium lauryl sulphate are widely used reference toxicants in the standardized LC50-assays using Brachydanio rerio, Daphnia magna and Artemia salina. The hexavalent chromium ion (in dichromate) is a strong oxidizing agent and readily reacts with organic matter thereby being reduced to the trivalent form. In its trivalent form chromium is strongly associated with proteins, nucleic acids and a variety of low molecular weight ligands (National research council, Commitee on biologic effects of Atmospheric Pollutants, 1974). Inorganic mercury compounds are rapidly biotransformed into shortchain alkyl mercury compounds. Practically all mercury in marine as well as freshwater fish is under the form of methyl mercury (Friberg and Vostal, 1972). Most mercury compounds bind to plasma proteins and are thus

transported within organisms (Suzuki, 1977). Mercury ions bind to thiol and carboxy groups of many proteins (Suzuki, 1977). All mercury compounds have some effect as C-mitotic agents (inhibiting mitosis) (Suzuki, 1977). Cesium has depolarizing effects on cell membranes and causes changes in intracellular cation composition (Luckey and Venugopal, 1977). Selenium mainly interacts with sulfhydril groups of the aminoacids cysteine and methionine and forms seleniumbridges between sulfhydrilgroups in proteins (Comittee on medical and biological efffect of environmental pollutants, 1976). 2-acetamidofluorene is a known procarcinogen that is metabolized by the mixed function oxidases in rats. Its metabolites are known to cause double strand breaks in DNA as well as the inhibition of RNA polymerase I and II activity (Hodgson and Guthrie, 1980). Phenacetin and 4-aminobiphenyl are a putative and known carcinogen respectively (National Toxicology Program, 1981).

The sensitivity of the D. bruciei or M. microphthalma assay system can not be directly compared to that of the existing simple ecotoxicological tests, since the criteria and protocols used for the detection toxicity are so widely different between test systems. The LC-50 (24 hrs.) for Artemia salina, Daphnia magna and Brachydanio rerio are 38, 1.42 and 301.7 mg/l respectively for potassium dichromate and 22.5 mg/l, 0.9 - 1.5 mg/l and 7.5 mg/l respectively for sodium lauryl sulphate (Vanhaecke and Persoone, 1979). All moults of D. bruciei are inhibited at a concentration of 0.56 - 1 mg/l potassium dichromate and all moults of M. microphthalma are inhibited at a concentration of 0.1 mg/l sodium lauryl sulphate.

Since the toxicity of some of the tested chemicals has been determined using the Panagrellus redivivus assay system, some comparison of the

sensitivity of the different "nematode assays" is possible (Table XII.). The nematode test using Diplolaimelloides bruciei (Lynher strain) detects toxicity at lower concentrations of 2, but higher concentrations of 3 of the 5 toxicants tested, than the nematode test using Panagrellus redivivus. At a concentration of 10^{-6} molar mercuric chloride only the P3-value of P. redivivus is reduced, while the fourth moult of D. bruciei is already significantly inhibited at a concentration of 10^{-7} molar and a high mortality as well as complete inhibition of all moults of D. bruciei are observed at a concentration of 3.2×10^{-6} molar mercuric chloride. Methyl mercury chloride causes 100% mortality of D. bruciei at a concentration of 3.2×10^{-8} molar, while a concentration of 10^{-6} molar methyl mercury chloride is required to cause significant lethality of P. redivivus. The third and fourth moult of P. redivivus are inhibited at a concentration of 10^{-8} molar methyl mercury chloride. The development of D. bruciei is at this concentration not affected. A concentration of 10^{-5} molar 2-acetamidofluorine causes significant lethality and decreases all P-values in D. bruciei (Lynher strain). This concentration of 2-acetamidofluorine is stimulatory to the development of P. redivivus. Exposure to a concentration of 10^{-3} molar phenacetin causes no mortality, but strong developmental inhibition of both P. redivivus and D. bruciei. The nematode test using P. redivivus detects strong inhibition of the third and fourth moult at concentrations of 10^{-3} to 10^{-8} molar. The nematode test using D. bruciei detects a distinct, but statistically non-significant, inhibition of the fourth moult at a concentration of 10^{-4} molar phenacetin. A concentration of 10^{-5} molar of 4-aminobiphenyl is semilethal to and reduces all P-values of both P. redivivus and D. bruciei. Lower concentrations (up to 10^{-8} molar), however, reduce P1 and P3 of P. redivivus,

but are not toxic to D. bruciei. At a concentration of 10^{-4} molar 95% of D. bruciei and 100% of P. redivivus are killed.

P. redivivus is more sensitive to mercuric chloride and cesium chloride than M. microphthalma. The P3-value of P. redivivus is reduced at a concentration of 10^{-6} molar mercuric chloride. Mercuric chloride is at this concentration not toxic to M. microphthalma. A concentration of 3.2×10^{-5} molar, however, is 100% lethal to M. microphthalma, while a concentration of 10^{-5} molar mercuric chloride is semilethal and inhibitory to the last three moults of P. redivivus. The development of P. redivivus is inhibited by 10^{-5} molar cesium chloride. Exposure to 10^{-3} molar cesium chloride has no effect on the development of M. microphthalma. A high mortality and a strong inhibition of development of M. microphthalma are observed at a concentration of 3.2×10^{-3} molar cesium chloride. Selenium oxide causes 47% mortality and a reduction of P2 and P3 in M. microphthalma at a concentration of 10^{-5} molar. At this concentration no mortality, but a reduction of P2 and P3, are observed in P. redivivus. Total mortality of M. microphthalma and no significant mortality, with a reduction of P2 and P3 of P. redivivus are observed at a concentration of 10^{-4} molar selenium oxide. On the other hand a reduction of P2 and P3 of P. redivivus is observed at a concentration of 10^{-8} molar, while a concentration of 10^{-7} molar is no longer toxic to M. microphthalma.

The Mexican strain of D. bruciei is less sensitive to toxicants than the Lynher strain (Tables VIII and IX). An increase in sensitivity to toxicants of D. bruciei was observed over a period of 9 months (Table VIII, third experiment with mercuric chloride; Table IX, second experiment with 2-acetamidofluorine). This could be due to the fact that at the onset of

this study our laboratory strains were not yet isogenic and that during the course of this study increasingly more inbred nematodes were used in the toxicity tests. This stresses the importance of using stable, inbred, isogenic laboratory strains in toxicity testing. The fact that long term cryogenic storage of strains of both D. bruciei and P. redivivus is possible is, in this respect, an added advantage.

D. bruciei is a more convenient laboratory organism than M. microphthalma, since it is easier to manipulate, grow in liquid growth medium and measure. Its development, furthermore, can be entrained to environmental stimuli in a manner similar to that of P. redivivus.

The D. bruciei assay system represents a rapid and cost-effective tool to quantitatively detect multiple biotoxic effects of environmental agents on a representative of a taxon that occupies a key position in the marine meiobenthos. Considering its similarity to the Panagrellus assay system, the D. bruciei nematode test has potential as a biomonitoring assay to detect the primary sites and sources of biotoxicity in marine ecosystems. Further testing of known chemicals, however, is required to validate the D. bruciei nematode test and to establish whether a specific block of the L4-adult moult of D. bruciei can be correlated with the phenotoxic action of an agent as is the case in the Panagrellus test. The flexibility of the D. bruciei assay system to the nature of the samples that can be tested also remains to be determined.

The nematode test using Panagrellus has been successfully used to determine the relative biotoxicity of extracts of complex environmental samples of a wide range of different compositions and origins (mine tailings, fish tissue homogenates, river sediments, ureaformaldehyde).

There are, therefore, good reasons to assume that the Panagrellus test could be used to determine the relative biotoxicity of extracts of complex environmental samples of marine origin. However, the Panagrellus test can not be directly applied in cases where it is required that an extract of a sample of marine origin be tested in a solution with an ionic composition identical to that of seawater. This is the only drawback to the use of the P. redivivus test as a "yardstick-test" (Samoiloff and Wells, 1984) to detect and map the primary sites and sources of biotoxicity in a marine ecosystem. Heip et al (1978) reported good growth of P. redivivus in natural seawater with a salinity of 17 - 23 pro mille. The use of a P. redivivus strain that can be grown in a culture medium with an ionic composition similar to that of seawater represents an alternative route towards the development of a yardstick biomonitoring assay for the marine environment.

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