Respiration Responses of a *Caenorhabditis* sp. and *Aphelenchus avenae* to the Nematicide, 1,2-dibromoethane (EDB)[1]

C. F. MARKS[2]

Abstract: The respiration rate of third stage larvae of *Caenorhabditis* sp. exposed to 0.53×10^{-2} M EDB was 120% greater than in untreated checks and was highest shortly after the exposure began. Similarly-treated third- and fourth-stage *Aphelenchus avenae* exhibited no marked respiratory response. Different responses of these animals to EDB probably reflect basic physiological differences between the nematodes. *Key Words: A. avenae, Caenorhabditis* sp., EDB, Nematicide, Respiration.

The toxicity of different nematicides and the differential reactions of various species of nematodes to them generally have been explained in terms of cuticular barrier effects (2, 4, 5). Marks, Thomason and Castro (6), however, demonstrated that organic-halide nematicides [such as 1,2-dibromoethane (EDB) and 1,2-dibromo-3-chloropropane (DBCP)] readily penetrated the cuticle of various species of both plant-parasitic and free-living nematodes, and established a dynamic equilibrium between the internal and external medium. This would indicate that the differential toxicity of organic-halide nematicides must result from differing intracellular effects.

Moje (7) hypothesized the mode of action of organic-halides in nematodes to be a displacement reaction with nucleophilic centers in one or more of the essential enzyme systems. Morikawa demonstrated EDB inhibition of triosephosphate dehydrogenase in the American cockroach (9) and *Panagrellus redivivus* (10) and suggested this might be its mode of action. Castro (1) suggested halogenated hydrocarbon activity may involve the halide oxidation of iron centers in the electron transport chain. It seemed desirable as a preliminary step in a study of the mode of action of EDB to determine its gross effects upon nematode metabolism by monitoring respiration. The primary objectives of the present work were to determine whether the organic-halide nematicide, EDB, affects the respiration of nematodes and, if so, to measure the correlation between the penetration of EDB and the respiratory response.

METHODS AND MATERIALS

Populations of *Aphelenchus avenae* Bastian and a *Caenorhabditis* sp. [designated earlier (3, 6) as *Pelodera* sp.] were reared as previously described (6) except that the cultures were maintained at about 22 C. Petri dish cultures of *Caenorhabditis* sp. were floated on a 3-mm layer of water in sealed polyethylene bags. Nematodes that migrated into the water during the first 72 hr were discarded and only the larvae collected in the two succeeding 72 hr floatation periods were used. Immediately after each collection the nematodes were allowed to pass through two 3-ply facial tissues (modified pan technique (11)) for about 12 hr. Thus only the more active larvae were retained. Agar containing *A. avenae* and fungus mat was placed on a tissue as described above and the nematodes were collected after 24 hr. The populations of both species of nematodes were screened through 100-mesh (147μ) and 200-mesh (74μ) sieves. Only those nema-

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[1] Contribution No. 190; Research Station, Canada Department of Agriculture, Box 185, Vineland Station, Ontario, Canada. The technical assistance of Mr. O. Sorensen is gratefully acknowledged.

[2] Nematologist, Canada Department of Agriculture, Box 185, Vineland Station, Ontario, Canada.

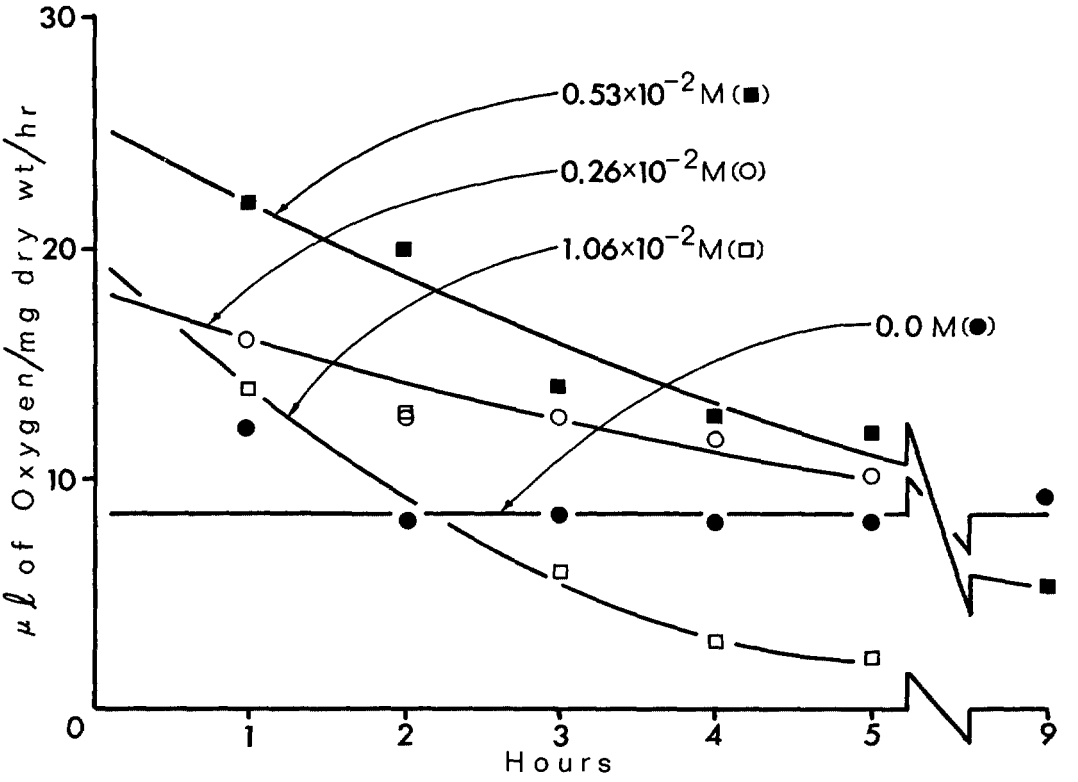

FIG. 1. Respiration rates of *Caenorhabditis* sp. exposed to different concentrations of EDB.

todes retained on the 200-mesh sieve were used in the experiments. This sieving procedure gave a practically pure population of third-stage larvae of *Caenorhabditis* sp. and a mixture of third- and fourth-stage larvae with a very small number of *A. avenae* adults. The nematodes were then washed several times with tap water on a 5μ Millipore® (Millipore Filter Corp., Bedford, Mass.) filter, and brought to the desired concentration. Nematode suspensions were used immediately after 1 hr of aeration. The *Caenorhabditis* sp. and *A. avenae* were, respectively, 13–85 and 1–25 hr away from the culture at the time of use. Nematode dry weight determinations were made after drying 72 hr at 85 C in a forced air oven.

The nematicidal chemical, 1,2-dibromoethane (EDB) used in this study was a Fisher "Certified" reagent.

Respiration was measured with a Warburg constant-volume respirometer oscillating at 110 strokes per minute. Flask volumes were approximately 21 ml at 250 mm on the manometers. Brodie's fluid (12) was used in the manometers. In all cases tap water was the suspending medium. The reaction flasks were equipped with two sidearms but no center well. One sidearm contained 0.2 ml of 10% KOH, the other contained 0.5 ml of EDB solution which was added to the nematode suspension after a 10-min equilibration period. The total liquid volume in each reaction flask was 3.2 ml. Treatments were replicated four times in each run and each run repeated at least three times. Pre-

liminary runs using EDB-control flasks showed that the vapor pressure of this chemical, at the low concentrations used in these experiments, did not influence manometer readings. Unless stated otherwise the bath temperature was 30 C.

Each reaction flask contained 25,000 *Caenorhabditis* sp. or 40,000 *A. avenae*. These numbers of nematodes contained 1.04 mg dry tissue and consumed easily detectable amounts of oxygen during 5–8 hr observation periods.

RESULTS

The respiration rates for the nematodes varied considerably from batch to batch but always followed the same trend for a particular treatment. Within any one run the variation between replicates was less than 10%. Since meaningful statistical measurements could not be applied to the data only selected runs that are representative of the results are presented in the figures below.

The respiration rate for *Caenorhabditis* sp. was highest when exposed to a 0.53×10^{-2} M concentration of EDB; 22 μl O_2 per mg dry wt per hr is 120% greater than the control rate. The respiration rates of the control nematodes remained relatively constant throughout a run while those of the EDB-treated nematodes followed the curves depicted in Fig. 1. Concentrations of EDB below 0.26×10^{-2} M produced no detectable respiratory change.

To determine whether the respiratory response of *Caenorhabditis* paralleled the permeation behavior of EDB (6) a number of nematodes were exposed to 0.53×10^{-2} M EDB for 1 hr, washed and resuspended in water. After 1 hr these nematodes were washed; half were resuspended in 0.53×10^{-2} M EDB and the remainder resuspended in water. The respiration rate of the nematodes resuspended in the EDB solution reached a maximum about 100% higher than

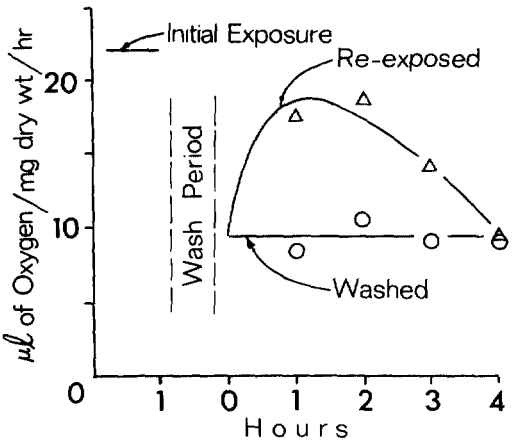

FIG. 2. Respiration rates of a *Caenorhabditis* sp. initially exposed to 0.53×10^{-2} M EDB, washed, then re-exposed to 0.53×10^{-2} M EDB.

that of the nematodes resuspended in water (Fig. 2).

The effect of EDB on the respiration of *Caenorhabditis* sp. was independent of temperature (Fig. 3) between 10 and 30 C. The Q_{10} ratio (respiration rate at temp + 10 C divided by respiration rate at temp) for respiration was 2.1 between 10 and 20 C and 1.4 between 20 and 30 C. These values were not altered when the nematodes were exposed to EDB. Also the respiratory quotient (RQ) for both the EDB-treated and the control nematodes remained relatively constant at 0.7 over the range of 10–30 C.

None of the concentrations of EDB tested evoked significant respiratory response in *A. avenae* (Fig. 4). A comparison of the results obtained with *Caenorhabditis* sp. (Fig. 1) and *A. avenae* (Fig. 4) demonstrate the marked difference in the response of these nematodes to the presence of EDB. In fact EDB depressed the respiration of *A. avenae*. The reaction of *A. avenae* to EDB was not affected by temperature between 10 and 30 C. In view of the lack of response of *A. avenae* to EDB, no attempt was made to determine RQ or the Q_{10} for respiration between 10 and 30 C.

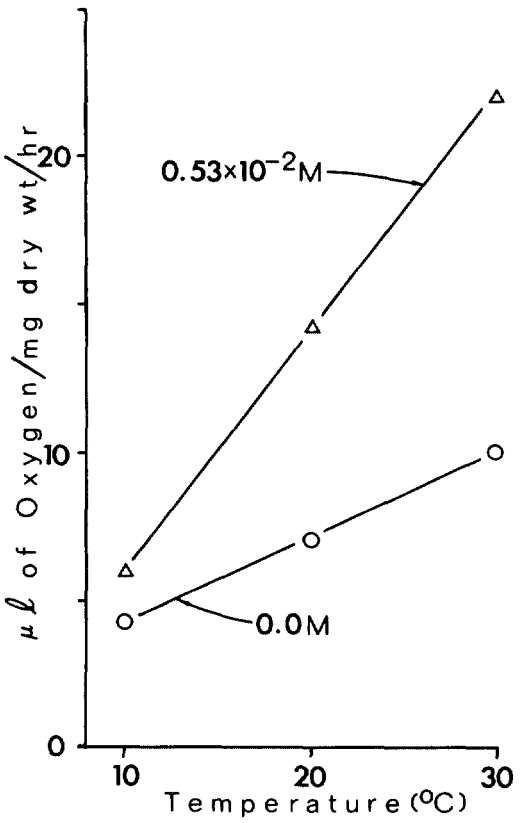

FIG. 3. Effect of temperature on the respiration rate of a *Caenorhabditis* sp. exposed to 0.0 M and 0.53 × 10^{-2} M concentrations of EDB.

DISCUSSION

The results of this study demonstrate that EDB has a stimulatory effect on the respiration of third stage larvae of *Caenorhabditis* sp. conforming with Morikawa's (9) observations on EDB-treated cockroaches. The respiration rate of *A. avenae*, however, was not significantly influenced by the presence of EDB. Since EDB penetrates both of these animals quite readily, the difference in the respiration response probably indicates basic physiological differences between species.

The present data suggest the mode of action of EDB in *Caenorhabditis* sp. differs from that postulated by Morikawa (10) for *P. redivivus*. A change in RQ would be expected if phosphoglyceraldehyde dehydrogenase were inhibited in nematodes utilizing a carbohydrate energy source. No change in RQ was observed in EDB-treated *Caenorhabditis* sp. This was not unexpected however since an RQ of 0.7 indicated these nematodes were surviving on stored lipids. It cannot be assumed that EDB did not inhibit phosphoglyceraldehyde dehydrogenase; however, the results demonstrate that the chemical affected other biochemical systems. Inhibition of phosphoglyceraldehyde dehydrogenase, therefore, may be of minor importance to the killing action of EDB in plant parasitic nematodes which generally use stored lipid as an energy source during the soil phase of the life cycle. On the other hand, Castro's hypothesis (1) of reversible oxidation of iron centers by bromide is supported by the respiration rates returning to normal when the EDB was washed from the nematodes and a second stimulation of respiration occurring upon re-exposure to the chemical. The lack of a EDB response by *A. avenae* indicates, however, that a generalized mode of action for a given type of nematicide, or even a given nematicide, cannot be assumed. On the contrary, the mode of action of a chemical may vary considerably depending upon the physiological differences between species of nematodes.

The absence of temperature effects upon respiration of *Caenorhabditis* sp. exposed to EDB, similar RQ and Q_{10} values for the respiration of treated and untreated nematodes between 10 and 30 C, and the reversibility of EDB effect on the respiration rate, suggest a physical rather than a biochemical mode of action. The major effect of EDB on *Caenorhabditis* sp. may be narcotic rather than an irreversible reaction of the type suggested by Moje (7).

The most surprising aspect of the present studies was the immediate respiratory re-

sponse of *Caenorhabditis* sp. to EDB. A less abrupt response paralleling the gradual increase in the internal concentration of EDB (6) might have been expected. The lack of correlation between the time required for the attainment of a maximum internal concentration of EDB and the onset of a respiration response indicates these two parameters are not related. The results can, however, be interpreted on the basis of the data of Marks et al. (6) which give the time required for permeation of the whole animal while in the present work the response time-lag reflects the time for the chemical to penetrate to the sites of action, possibly the cells of the body wall. If this is true, then the rate-limiting step in the overall permeation process for EDB as described by Marks et al. (6) must be at some point beyond the hypodermal cells, for example the movement of EDB from these cells into the pseudocoelom; diffusion across the pseudocoelom; or movement into the intestine. The immediate response of *Caenorhabditis* sp. further demonstrates that the cuticle does not appreciably impede the permeation of EDB.

Since the full effect of the chemical is observed immediately upon exposure, there must be a threshold concentration of EDB required to evoke a respiration response. This threshold concentration is about 0.26×10^{-2} M in the suspending medium. We have, however, no idea of the concentration of the chemical at the site of action. The highest concentration of EDB in the external medium, 1.06×10^{-2} M, appeared to have a deleterious effect on the nematodes.

The results presented in Fig. 3, combined with the data of Marks et al. (6), which showed that EDB could be readily washed out of nematodes, demonstrate that the respiration response of the *Caenorhabditis* sp. is directly related to the presence of EDB in the nematodes. This relationship, however, does not prove a direct correlation between the respiration response and the lethal action of EDB.

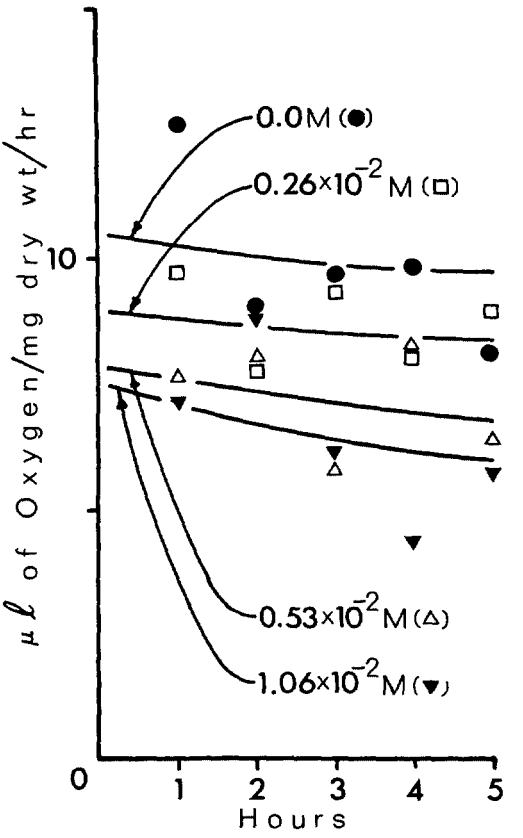

FIG. 4. Respiration rates of *A. avenae* exposed to different concentrations of EDB.

The validity of respiration data on a bacteriophagous nematode such as a *Caenorhabditis* sp. might be questioned because of the possibility of bacterial contamination, but this is highly improbable in this work since: (i) the nematodes were thoroughly washed to remove surface bacteria; (ii) the water collected from the nematode washes, did not show a detectable O_2 consumption even over long periods of time; (iii) massive populations of bacteria collected from the cultures actually showed a decrease of about 50% in oxygen consumption when exposed to

0.53×10^{-2} M EDB; and (iv) surface-sterilized nematodes placed on nutrient agar did not show bacterial contamination associated with the animals indicating that there were no viable bacteria in their intestines.

It is surprising that the *Caenorhabditis* sp. and *A. avenae* should react so differently to a general biocide such as EDB. Since the respiration response of the *Caenorhabditis* sp. to EDB was not associated with any visible increase in motility apparently the respiratory physiology, or some key enzyme(s) in a pathway(s) that affects oxygen consumption differs markedly from that of *A. avenae*. Although this study does not clarify the mode of action of EDB it does illustrate that conclusions based on results obtained from one species of nematode or from the measurement of one parameter are tenuous at best. It also indicates a possible pitfall in hypothesizing a generalized mode of action for halogenated hydrocarbon nematicides. It is apparent that more information is needed on the biochemistry of nematodes, at least the more important plant parasitic species, before further major advances in our understanding of the mode of action and selectivity of nematicides can be made.

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