

Toxicological and Physiological Effects of DDT on *Caenorhabditis elegans*

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Abstract

Toxicity assays were used in this study to test how DDT affects lethality and brood size of *Caenorhabditis elegans* (*C.elegans*) by exposing them to various concentrations of this agent. These nematodes have provided a very informative system that was utilized to study the behavioural and physiological processes. The results showed that DDT affected the lethality in a dose-dependent manner, but 100% kill was not achieved with concentration tested. Whereas, sodium azide, positive control does have an effect on *C. elegans* and significantly inhibiting lethality (LC₅₀ 0.01mM).

Similarly, DDT led to a pronounced effect in brood size of *C.elegans* compared to the mean brood size recorded for the control (0.1% DMSO). Sodium azide results showed a greater difference in brood size compared to DDT.

Both agents, DDT and sodium azide caused a remarkable inhibitory effect on *C.elegans* pharyngeal pumping rate.

It can be concluded that the target site of DDT in *C.elegans* might not be the same target in insect.

Key words : DDT – Sodium azide - *Caenorhabditis elegans*

Introduction

The long term use of many insecticides is continually threatened by the ability of insect to evolve resistance mechanism that renders the chemical ineffective [1] DDT has had a significant impact in the control of malaria and other insect diseases, and was extensively used as an agricultural insecticide after the Second World War . This agent was found to have a chronic effect on the nervous system, liver, kidney, and immune system in experimental animals . [2]

Caenorhabditis elegans (*C.elegans*) has been extensively utilized as a model animal for the study of nematode behavioural and physiological processes [3]. *C.elegans* has also proven itself a model of biological studies relevant to higher animals in such areas as neuroscience, genetics, aging, and development. Thus, toxicological results in *C.elegans* are likely to higher animals [4]. However, one tissue that has proven more amenable to physiological analysis is the pharynx. This is a muscular pump which sucks bacteria into its lumen, grinds them and then passes them to the gut [5]. This provided insight into the mode of action of toxins and

how *C.elegans* responds to protect itself from toxin. This has contributed and will continue to contribute to our biological understanding of all animals including human.

In the present work an attempt has been made to investigate the effect and the mode of action of DDT in order to probe the toxicity and the accumulation of this pesticide. Until

recently little is known about the pharmacological effect of this insecticide and how it works on *C. elegans* which is used as model organism in this study.

Materials and Methods

C.elegans were cultured on agar and fed with *E.coli* (OP50) throughout this study. Worms were grown at 15⁰ C on NGM plates containing (NaCl 3g, Agar 17g, Peptone 2.5g, and 1ml Cholesterol (5mg/ml ethanol) in 1L).

For the lethality assay 50 L₄ *C.elegans* N₂ worms per dose group were placed in 400ul of liquid medium (S-basal) supplemented with 40ul *E.coli* (O.D 0.7 600nm) with varying concentrations of DDT or sodium-azide (NaN₃) to each well of 24-well plate [6]. The 24-well plate was then sealed with a laboratory film (to prevent evaporation). Wrapped in a damp paper towel (to provide humidity) and placed in an air tight plastic container and incubated for 3 days. After the incubation period, the percentage of live worms were determined by counting the number of viable and dead worms in each well based on movement.

To measure the effects of DDT on brood size, the assay was set up in 48 well-plates, with 4 well for each concentration. An L₄ worm was placed in each well with 40ul of bacteria culture, the toxin and s-basal to make a total volume of 400ul. The 48 well-plate was then sealed and placed in an air tight plastic container and incubated at 15⁰C for 3 days. After 3 days the number of offspring in each well was counted under a stereo microscope. An identical assay was set up and run alongside with sodium azide (as positive control).

Pharyngeal pumping assay was set up the same as the lethality bioassays. Briefly, the worms were exposed to various DDT or sodium-azide concentrations for an hour, before the worms transferred to NGM plates and left for another an hour to settle down before the pharyngeal pumping rate was counted.

Reagents: All solutions were made up to 1 litre with UHP water and sterilized by autoclaving storage was at room temperature.

DDT stock solution made up to 10 mg/ml in DMSO (Dimethyl sulfoxide) this was serially diluted to make 1 ml of each concentration used in this investigation.

Results

C. elegans has been used as a model organism in order to probe toxicity and the accumulation of pesticides as well as various metal ions. In this study *C.elegans* was used as a system to gauge the toxicological effects of DDT and sodium azide.

- Lethality assay

50 L₄ larvae were exposed up to 5ug/ml for 3 days. The experimental results showed that DDT had marked inhibitory effect at 5ug/ml, but little effect at 1ug/ml (fig.1). Lethality was affected in a dose-dependent manner, but 100% kill was not achieved with concentration tested.

Sodium-azide was also employed as positive control due to its well known toxic effect [7]. The results obtained demonstrated that exposure of *C.elegans* to the respiratory inhibitor sodium azide, this agent caused a remarkable inhibitory effect on *C.elegans* lethality and the

LC₅₀ was 0.01mM (fig2). The graph represents a single experiment with each concentration tested in triplicate to produce standard error (represented by bar) and mean percentage of viable worms (presented by dots)

- Brood size assay

Individual L₄ *C.elegans* were placed in 400ul of liquid medium with varying concentrations of DDT, incubated for 3 days at 15⁰C the effect was determined by counting the offspring in each well. It was found that exposure of the worms to (0.001ug/ml-1.0ug/ml) DDT led to a pronounced effect in the brood size of *C.elegans* (fig.3). The mean brood size recorded (41.6) for the control (0.1% DMSO).

In parallel with DDT sodium azaide was tested as a positive control, the results showed that sodium-azide has an effect on *C.elegans* and inhibits brood size. The maximum brood size recorded was 30.5 and the greatest inhibition was at 0.01mM. There was significant difference in brood size seen for the concentration of sodium azide tested (fig.4)

-Pharyngeal pumping assay

The pharynx of *C.elegans* has several features that make it suitable for cellular and molecular studies of behaviour. This model system has been studied by observing its behaviour in normal worms and treated worms. Pharyngeal pumping is the coordinated and regulated process by which the pharynx mediates intake and forces the food and waste products through the worm gut [8]. The results presented here showed that DDT does have an inhibitory effect on *C.elegans* by decreasing the pumping rate in a dose-dependent manner. These results summarized graphically in fig5. The pharyngeal pumping is also reduced in response to sodium azide; however, the higher concentration of sodium azide stopped the pharynx activity in some worms (fig.6).

Discussion

The exposure of animals to exogenous agents has been of considerable value in analysing *C.elegans* response. Most of pharmacological agents that have been used in *C.elegans* affect various aspects, such as brood size, lethal concentration, and growth measurement. DDT is an organochlorine insecticide used mainly to control insect typhus and malaria vectors. This agent has been banned for use in different countries, although it is still used in other. There is evidence that DDT causes reproductive effects in test animals [2] and this seems to agree with results obtained in this study because DDT had a dose-dependent inhibitory effect on the brood size of *C.elegans*. Insecticides may act on membrane proteins (receptors, channels) increasing the ability of the insect to detoxify the insecticide or by changes in the target protein with which the insecticide interacts [1]. DDT is an insect neurotoxin that interferes with ion flow regulation across the sodium ion channels [9,10] increasing the flow of sodium ions via sodium channels, thus, the channels are kept open and there is prolonged inward conductance of sodium causing repetitive nerve firing which can lead to paralysis and death of the insect [11,10]. This may not be the story in *C.elegans* because patch-clamp experiments on *C.elegans* neurones [12] and body wall muscle cells [13,14] showed that no

sodium current was observed, furthermore, intracellular recordings from pharyngeal muscle cells have shown a dependence spike generation on external sodium [15]. Moreover, the muscle cell's of the pharynx communicate with each other via a network of gap-junction made up of innexins this confers a high degree of electrical connectivity which likely to play a central role in coordinating waves of myogenic excitation and the response to neuronal modulation [5]. [16] found that, although the pharyngeal pumping rate is under the control of the neurones, the pharynx continues to pump even when the pharyngeal neurones have been ablated, providing evidence that the pharynx may be myogenic. Further investigation (intracellular recording) might be needed to find out the target site of DDT which cannot be the same target as in insects.

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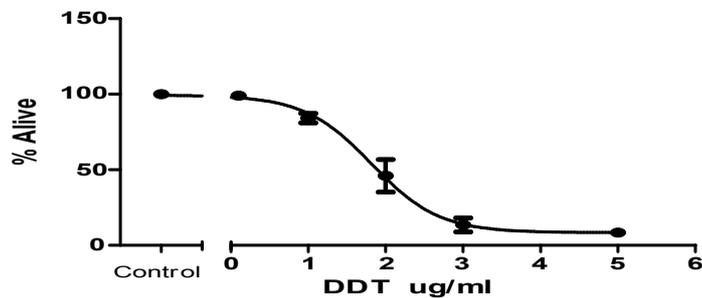


Fig.(1) Lethal concentration assay. This graph depicts a single LC_{50} in which the number of worms live after 3 day of exposure to dose DDT (0.1ug/ml–5.0 ug/ml) or vehicle control (0.1% DMSO) L_4 *C.elegans*. The graph represents a single experiment with each concentration tested in triplicate where $n=50$, $t=15^0$ C. This experiment was repeated with similar results.

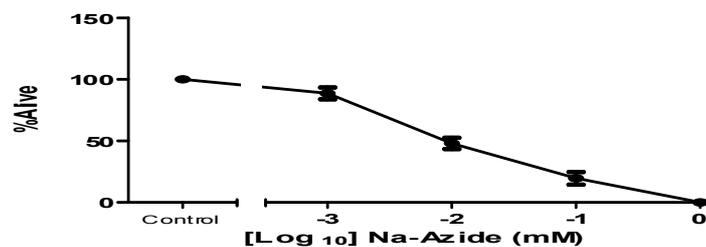


Fig.(2) Lethal concentrations assay of sodium azide (0.0001mM-1.0mM). This graph was set up as described in figure: 1 as appositve control.

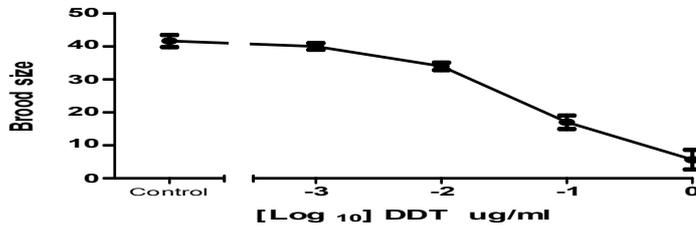


Fig.(3) Effect of various concentrations of DDT (0.001ug/ml-1.0ug/ml) or vehicle control (0.1% DMSO) on brood size of L₄ *C.elegans* N₂ worms, where n=4, t=3days at 15⁰ C. This experiment was repeated giving similar results.

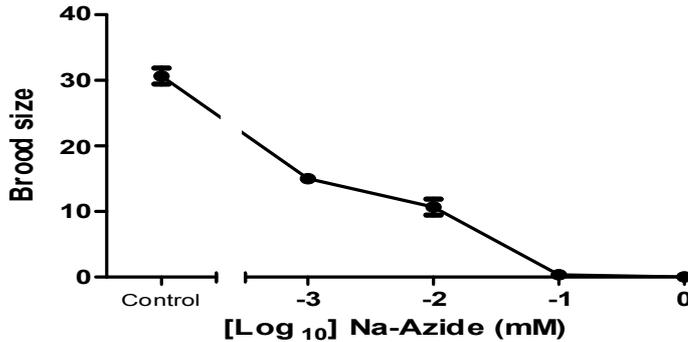


Fig.(4) Effect of various concentrations of sodium azide (0.0001mM-0.1mM) on brood size. This experiment was set up as in figure 3 as a positive control.

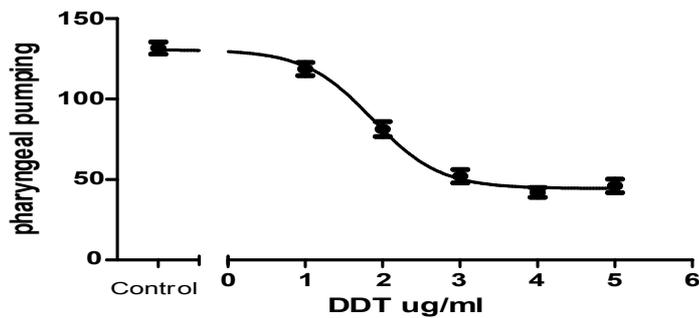


Fig.(5) Effect of various concentrations of DDT (1ug/ml-5ug/ml) or vehicle control (0.1%DMSO) on pharyngeal pumping rate after one hour of exposure where n=6.

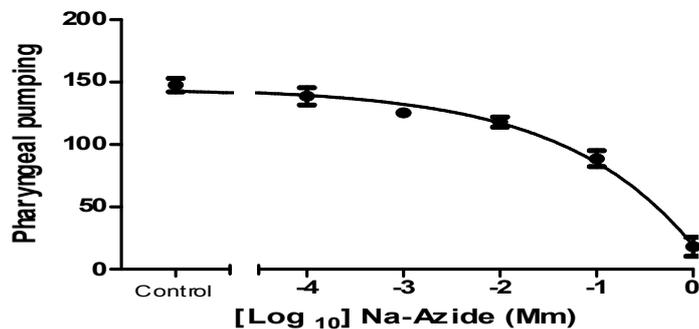


Fig. (6) Effect of various concentrations of sodium azide(0.0001mM-1.0mM). This experiment was set up as describe in figure 5 where n=6.

التأثيرات السمية والفسیولوجية لل DDT في *Caenorhabditis elegans*

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الخلاصة

استعملت اختبارات السمية في هذه الدراسة لمعرفة مدى تأثير الـ DDT في تحديد الجرعة القاتلة وحجم الحضنة الواحدة في ديدان *Caenorhabditis elegans*

(*C.elegans*) وذلك من خلال تعريضها لتراكيز مختلفة من هذا المبيد .

ان هذه الديدان وفرت معلومات قيمة استخدمت في دراسة العمليات الفسيولوجية والسلوكية المختلفة .

وقد اظهرت نتائج هذه الدراسة ان تأثير الـ DDT كان معتمداً على التركيز في تحديد الجرعة القاتلة ولكن دون الوصول الى تأثير قاتل 100% في جميع التراكيز المستعملة على عكس Na - azide الذي كان له تأثيراً معنوياً اذا كان الـ LC₅₀ عند التركيز 0.01 ملي مول .

وبطريقة مشابهة فقد اثر الـ DDT في حجم الحضنة الواحدة لديدان الـ *C.elegans* مقارنة بمجموعة السيطرة (0.1 DMSO) وكان التأثير Na- azide اكثر وضوحاً من الـ DDT

كلا المركبين DDT و Na- azide سببا تثبيطاً واضحاً في معدل ضخ العضلات البلعومية .

ومن خلال هذه النتائج يمكن الاستنتاج ان الموقع الذي يستهدفه الـ DDT في (*C.elegans*) لا يمكن ان يكون هو الموقع نفسه الذي يستهدفه في الحشرات .

الكلمات المفتاحية: دي دي تي، صوديوم أزيد ، *Caenorhabditis elegans*