



Journal homepage: www.sciencearchives.org

ISSN: 2582-6697



Original Research Article

<http://dx.doi.org/10.47587/SA.2021.2109>



An optimized assay for early and rapid assessment of behavioral alterations using *Caenorhabditis elegans* as an alternate animal model

Sarita Mishra, Rakhi Agarwal*

Laboratory of Analytical & Molecular Toxicology (Forensic Chemistry & Toxicology Laboratory)
School of Forensic Science, National Forensic Sciences University, Gujarat, India

*Corresponding author: rakhi.agarwal@nfsu.ac.in

Received: Jan 25, 2021 / Revised: Feb 25, 2021/ Accepted: Mar 22, 2021

Abstract

Exposure to various xenobiotics and microbial infections raise serious health concerns including the several phenotypic and behavioral anomalies in the exposed animals. Thus, an early assessment of altered behavioral endpoints will help to identify the toxic nature of a xenobiotic compound and its adverse impacts on the living organism. Several model organisms are being used for the toxicity assessment of various xenobiotics. *C. elegans*, a worm, possess several behaviors such as pharyngeal pumping, locomotion (speed, body bending), etc. Thus, by examining the alterations in such parameters, one can easily assess the behavioral abnormalities associated with toxicants/infections. In the present study, we have demonstrated a stepwise, simplified version of pharyngeal pumping and the locomotion assays (speed, body bending, omega turn, and reversal locomotion behavior) that can be easily performed in the laboratory for the early health assessment.

Keywords Rapid Assessment, Early Biomarker, Toxicant exposure, Alternate Model, *C. elegans*, Locomotion, and Pharyngeal pumping behavior.

Introduction

Behavior is the way by which animals respond to stimuli (Hart, 2006). It can be attributed to the search for food, to reproduce, to interact and protecting themselves, etc. It has been demonstrated that exposure to various health hazards/chemicals/toxicants adversely affects animal behavior (Hale et al., 2017). Thus, the study of the nature and impact of such toxicants on animal behavior is the key to address various health and safety issues. Several animal models like fruit flies, butterflies, fishes, earthworms, frogs, monkeys are being used to study the behavioral alterations associated with different agents/toxicants/ chemicals/drugs/nanoparticles, etc. *C. elegans*, a worm is used as an early biomarker to assess the impact of microbial infection (Kaletta and Hengartner, 2006; Marsh and May, 2012; Kong et al., 2014; Elkabti et al., 2018) and exposure of toxicants (Salim and Rajini, 2014; Kudelska et al., 2017; Mishra and Agarwal, 2020; Tiwari et al., 2020). Present work includes the systematic procedures and the role of pharyngeal pumping and locomotion assays for early assessment of behavioral alterations due to the exposure of

any xenobiotics. The different kinds of behavior shown by *C. elegans* include the followings:

Pharyngeal pumping behavior

Pharyngeal pumping behavior is rhythmic, alternate contraction and relaxation of the neuromuscular organ, pharynx which helps in the transportation of food towards the intestine through the pharynx (Avery and Shtonda, 2003; Trojanowski et al., 2016). The pharynx in *C. elegans* consists of 3 parts i.e. corpus, isthmus, and the terminal bulb with the grinder in it (Fig. 1A). Pharyngeal pumping in the worm *C. elegans* includes pumping (Fig. 1B) and peristalsis process (Fig. 1C) Pumping includes contraction of the corpus, anterior isthmus muscles, and the posterior bulb while contraction of corpus and anterior isthmus open the lumen to enter the food particles into it. Grinder plates move with the aid of posterior bulb contraction which initiates the breakdown of bacteria or food particles and moves it to the intestine. This is quickly

followed by relaxation of muscles which helps to expel out the trapped liquid from the lumen and food particle or trapped bacteria moves from posterior isthmus to the posterior bulb (Avery and Shtonda, 2003).

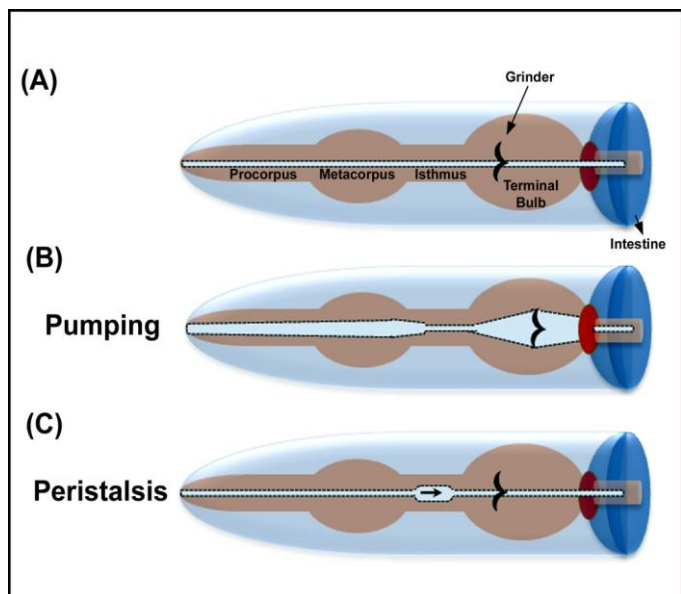


Fig. 1 Schematic diagram showing the anatomy of pharynx (A) and pharyngeal pumping behavior (B & C) in *C. elegans* including pumping and peristalsis

Locomotion Behavior

Locomotion Behavior in *C. elegans* is the coordinated behavior of dorsal and ventral muscles. With the release of acetylcholine neurotransmitter, propagation of sinusoidal waves takes place either in forward/reverse direction (Gjorgjieva et al., 2014; Zhen and Samuel, 2015; Maulik et al., 2017). The propagation of sinusoidal waves from head to tail results in forward movements while from tail to head result in reverse movements (Zhen and Samuel, 2015; Maulik et al., 2017). There are different types of locomotion exhibited by *C. elegans* varying in the form depending on the media in which *C. elegans* is incubated, i.e. head thrashing (Swimming in liquid medium) and crawling (on solid Agar). Further, endpoints like speed, body bending, reversal frequency behavior, and omega turn can be used to assess the locomotion behavior of *C. elegans*. These above-mentioned locomotion parameters can either be assessed manually with a compound microscope or can be assessed with worm tracking software (Hart, 2006; Salim and Rajini, 2014; Lu and Qiu, 2017). As mentioned, neurotransmitters like acetylcholine, serotonin, and glutamate are responsible for pharyngeal pumping and locomotion behavior (Avery and You, 2012, Gjorgjieva et al., 2014, Zhen and Samuel, 2015, Maulik et al., 2017). Therefore, pharyngeal pumping and locomotion assays are the important endpoint assays that can be used to assess the adverse effects of xenobiotics or toxicants on neuronal functions.

The details of different locomotion behavior in *C. elegans* are as below

Head Thrashing

Head thrashing is the type of behavior exhibited by *C. elegans* in the liquid medium. In this, *C. elegans* swim by showing lateral movements i.e. when the head tries to reach the tail either from the left side or right side, calls for the head thrashing behavior (Fig. 2A & B) (Buckingham and Sattelle, 2009; Kudelska et al., 2017).

Crawling

During crawling behavior on nematode growth medium (NGM), the worm exhibits different types of movements/gestures like body bending, reversal frequency, and omega turn. One body bend can be defined as the formation of an alternate longitudinal crest behind the pharynx followed by a longitudinal trough (Fig. 2D, E & F) (Hart, 2006). Speed of worm is the distance covered by *C. elegans* per min in the forward direction (Beron et al., 2015) while the formation of “Omega” shape by *C. elegans* is referred to as “omega turn” or deep bend (Fig. 2C) (Huang et al., 2006; Beron et al., 2015). Propagation of sinusoid waves from tail to head leads to reversal behavior in worm’s locomotion (Zhao et al., 2003; Hart, 2006; Beron et al., 2015).

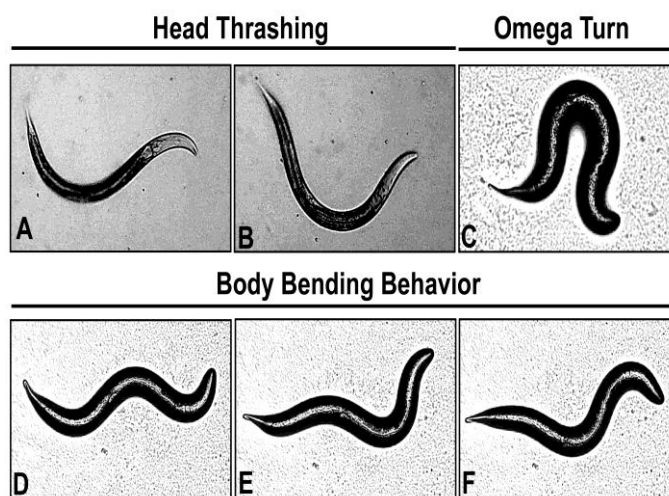


Fig. 2 Locomotion behavior in *C. elegans*. Image showing head thrashing behavior (A-B, one head thrash), omega turn (C), and body-bending behavior (D-F, One body bend)

The details of materials and methods to perform the said behavioral parameter are described below

Materials and Methods

1. M9 buffer
2. NGM Plate (Seeded and Unseeded)

Equipment

1. Laminar air flow (Thermoscientific, and HERAGUARD ECO, US)

2. Compound microscope (Micros, Austria)
3. Micropipettes (Thermoscientific)
4. 15mL falcon tube (Genaxy, New Delhi, India)
5. 2mL eppendorf tube (Genaxy, New Delhi, India)
6. 90×15 mm Petri plate (Genaxy, New Delhi, India)
7. 96 well plate for exposure (Genaxy, New Delhi, India)
8. Glass Slide (Bluestar, Mumbai, India)

Procedure

1. Nematode Growth Medium Plate (NGM Plate)- The NGM plate was prepared by adding 3g Sodium chloride, 17g Agar, 2.5g Peptone to 975 mL distilled water. It was mixed thoroughly and was autoclaved. Once the mixture was cooled to 55°C, 1mL of 1 M cholesterol (do not autoclave), 1mL of 1 M autoclaved CaCl₂, 1 mL of 1 M autoclaved MgSO₄, and 25 mL of 1 M autoclaved KH₂PO₄ (pH6) were added to it. The mixture was mixed thoroughly and poured exactly 22 mL aseptically into the Petri plates (Stiernagle, 2006). All the reagents required need to be autoclaved except cholesterol. NGM plates can either be seeded or unseeded depending on the presence or absence of *E. coli* culture.

2. *E. coli* culture for seeding NGM plate- Seeded plates are required for culturing and maintaining the *C. elegans* stock for synchronization, pharyngeal pumping assay, reproduction assay, egg viability assay, etc. For *E. coli* preparation we have added 0.75 mL of 1 M NH₄Cl (5.35 mg/mL in autoclaved distilled water), 0.75 mL of 20% glucose (200 mg/mL in autoclaved distilled water), 15 µl of uracil (2 mg/mL in autoclaved distilled water), the culture of 1.5 mL of OP 50 strain of *E. coli* as starter culture). The final volume was made up to 75 mL with the M9 buffer. It was mixed thoroughly and kept overnight at 37°C in an incubator shaker with continuous shaking at 200 rpm (Stiernagle, 2006).

3. M9 buffer M9 buffer was used for *E. coli* culture preparation, for taking worm washes, for synchronization, and also at the time of exposure of toxicant (to adjust the volume in the 96-well plate at the time of toxicant or test compound exposure). To prepare the M9 buffer, 3 g KH₂PO₄, 6 g Na₂HPO₄, 5 g NaCl, 1 mL of 1 M MgSO₄ was added to distilled water to make the final volume as 1 L. The mixture was mixed thoroughly and autoclaved. The buffer was cooled down and was stored at 4°C till further use (Stiernagle, 2006).

4. Synchronized *C. elegans* culture - As synchronization reduces the variation in result due to the age differences in worms, therefore it is recommended to have a synchronized population at the time of toxicant or xenobiotics exposure to the worm (Mishra et al., 2020). The synchronized worms were collected by selecting the NGM plate with maximum adults full of eggs. The worm wash was collected by adding 3.5mL of M9 buffer on the plate, swirl gently, and collecting the worm wash with cut tip micropipette in a 15 mL falcon tube.

0.5mL 5N NaOH + 1mL hypochlorite bleach was added and tapped gently for 7 mins. The mixture was centrifuged at 3410 rpm for 30 s at 22°C. The supernatant was removed and 5mL M9 buffer was added. The mixture was tapped gently for 2 mins and centrifuged at 3410 rpm for 30 s at 22°C. The supernatant was removed and the egg pellet was decanted on the unseeded plate. The worms (L1staged) were transferred on seeded plates after 10-12 hrs for further development (Mishra et al., 2020).

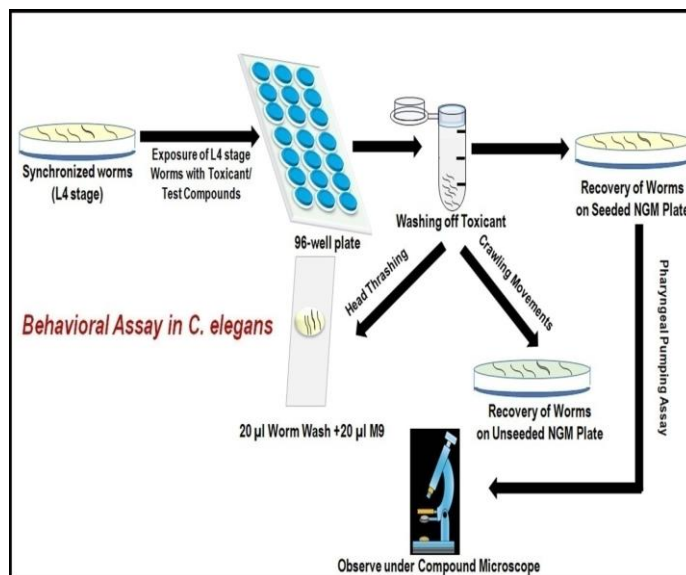


Fig. 3 Schematic diagram showing the stepwise protocol to assess the pharyngeal pumping and locomotion behavior in *C. elegans*

Step by Step Procedure

1. Select the NGM plate with maximum gravid (with full of eggs) hermaphrodite worms and synchronize (L1) it as described by Mishra et al. 2020.
2. Transfer the worms (L1 staged worms i.e. 10-12 hrs from synchronization) to seeded NGM plate and allow them to grow till the L4 stage (i.e. 28 hrs from the L1 stage at 22°C).
3. Collect the synchronized L4 worms using M9 buffer (worm wash) into a 2mL Eppendorf tube. The worm wash can be taken by tilting the synchronized plate (L4) at an angle of 45° and pouring 1 mL of M9 buffer (with the help of a 1 mL micropipette).

Note: Use of cut microtip is helpful to collect the desired worms.

4. Swirl gently to dislodge the worms and collect the worm wash with the help of a 1 mL micropipette (using Cut Tip).

Note: Don't shake the plate vigorously during collecting worm wash to avoid the dislodging *E. coli* along with worms.

5. Expose the worms in a 96-well plate with desired test compounds without *E. coli*.
6. After the exposure, wash off the test compounds by taking the contents of the well into the eppendorf tube with the help of 1 mL micropipette (Cut Tip) and add 1 mL of M9 buffer. Allow the worms to settle down by gravity and remove the supernatant (repeat this step thrice to completely remove the test compounds).
7. **For pharyngeal pumping assay** transfer the exposed worms onto the seeded NGM plate and allow acclimatizing for 1 min. Observe the pharyngeal pumping behavior under a compound microscope (Fig 1 and Fig 3). Pharyngeal pumping can be easily counted under a compound microscope using a 10X objective lens by observing the movements of the grinder carefully (Hart, 2006; Raizen et al., 2012).
8. **For head thrashing assay** take 20 μ L M9 buffer along with 20 μ L treated/exposed worms onto the slide. Allow the worms to get accustomed for 1 min and observe under a compound microscope using a 10X objective lens for the swimming behavior for the 20s (Fig 2 and Fig 3).
9. **For crawling behavior** place 20 μ L of treated/exposed worm wash onto unseeded NGM plate and allow the worms to acclimatize for 1 min. Examine the crawling behavior under a compound microscope (Fig 2 and Fig 3) and record the videos. The videos can be used for crawling behavior analysis manually or using WormLab software (3.0.0 Version, MBF Bioscience, Williston, VT, USA).

Software used

WormLab software (3.0.0 Version, MBF Bioscience, Williston, VT, USA, Trial Version <https://www.mbfioscience.com/wormlab>).

Discussion

The continuously increasing population has emerged a great challenge to provide food and health care to every individual. Thus, to fulfill the requirements of an ever-growing population, the growth of several agriculture, pharmaceuticals and cosmetics industries has been significantly increased. The compound synthesized or effluents released from various industries have adversely affected the ecosystem directly or indirectly and incurred a great challenge for the researcher to identify the toxic nature of a given compound at a very early stage. Early detection of toxicity associated with various compounds can be helpful to regulate them carefully the use of several chemicals/compounds based on their toxic nature. This toxicity assessment requires the use of thousands of animals/year. According to the “Annual Statistics of Scientific

Procedures on Living Animals Great Britain 2019” released by the Home Office, 432,500 animals were used in regulatory tests in 2019 in the UK alone (Annual Statistics of Scientific Procedures on Living Animals, Great Britain, 2019). Thus, to explore the toxicity associated with various chemicals/toxicants different non-mammalian animal models such as Zebrafish, Daphnia, *Drosophila melanogaster*, *C. elegans*, etc. are widely used now a days. The use of the vertebrate animal models have several financial and ethical constraints which demanded an alternative assessment means (Burden et al., 2015). *C. elegans*, a nematode has shown its potential for toxicological studies (Hunt 2017, Xiong 2017, Hunt et al., 2018; Keller et al., 2018, Ruszkiewicz et al., 2018). *C. elegans* has gained its popularity over other vertebrate model organisms (Wittkowski et al., 2019) due to its easy handling, easy maintenance (Raizen et al., 2012), only 302 neurons (Ruszkiewicz et al., 2018), availability of transgenic strains, RNAi and ~40% similarity with human disease genes (Culetto and Sattelle, 2000). Toxicity assessment using *C. elegans* has gained advantages over different analysis which uses the vertebrate animal model. These toxicity assessments using *C. elegans* are cost-effective, rapid, and do not require high-end instruments. In the current protocol, we have presented a stepwise description of different assays i.e. pharyngeal pumping and locomotion assay for the assessment of behavior toxicity in *C. elegans*. The identification of behavioral parameters is the key to address the neuronal problem. Having a comprehensive understanding of the nervous system is not an easy task but little can be explored by observing pharyngeal pumping and locomotion behavior in *C. elegans*. Behavioral parameters can be used as an early biomarker to assess the impacts of toxicants, pesticides, drugs, nanoparticles, any xenobiotics, etc. on the behavior of the animal at the initial stages of screening (Salim and Rajini, 2014; Salim et al., 2018; Mishra and Agarwal., 2020). Information obtained from the behavioral assessment can be further integrated with cellular, molecular and genetic approaches to obtain a complete understanding of effects of the desired compound on the nervous system. Behavioral parameters like speed, head thrashing, reversal frequency, omega turn and body bends have been used to assess the lead (Tiwari et al., 2020) and dichlorvos toxicity (Mishra and Agarwal, 2020) at sub-lethal doses in *C. elegans*. Heavy metals toxicity has been observed using behavioral parameters in *C. elegans* (Anderson et al., 2004, Ruszkiewicz et al., 2018). Assessment of dichlorvos pesticide and neurophysiological alterations due to the same in *C. elegans* has used pharyngeal pumping (Jadhav and Rajini, 2009). Pharyngeal pumping has also been used as an important parameter to assess the sublethal effects of dichlorvos on *C. elegans* (Jadhav and Rajini, 2009). Locomotion assay has received considerable importance in finding toxicity testing of monocrotophos during development in metabolic disorder condition such as high glucose fed *C. elegans* (Salim and Rajini, 2014). Further, the ameliorative function of curcumin on dopaminergic neuronal dysfunction in *C. elegans* also has examined by locomotion assay (Satapathi et al., 2016).

Thus, it was concluded that the behavioral assays are useful for low-throughput toxicity screening of any compound using *C. elegans*.

Acknowledgment

The authors are grateful to the Vice-Chancellor, and Campus Director, National Forensic Sciences University, Gujarat, India for providing all necessary facilities during the research work. Data included in the paper are the part of research for the award of a doctoral degree to SM.

Conflict of Interest

The author hereby declares no conflict of interest.

Funding support

SM acknowledges the Scheme of Developing High Quality Research (SHODH), Gujarat, India for financial assistance.

Ethics

The study was approved by University Research Committee (No. Ph.D./FS/RA/005).

References

Anderson, GL, Cole, RD, & Williams, PL (2004). Assessing behavioral toxicity with *Caenorhabditis elegans*. *Environmental Toxicology and Chemistry: An International Journal*, 23 (5), 1235–1240.

Avery, L., & Shtonda, B. B. (2003). Food transport in the *C. elegans* pharynx. *Journal of Experimental Biology*, 206(14), 2441-2457.

Avery, L., & You, Y. (2012). *C. elegans* feeding. In: The *C. elegans* Research Community (eds) *WormBook*. doi/10.1895/wormbook.1.150.1.

Beron, C., Vidal-Gadea, A. G., Cohn, J., Parikh, A., Hwang, G., & Pierce-Shimomura, J. T. (2015). The burrowing behavior of the nematode *Caenorhabditis elegans*: a new assay for the study of neuromuscular disorders. *Genes, Brain and Behavior*, 14(4), 357-368.

Buckingham, S. D., & Sattelle, D. B. (2009). Fast, automated measurement of nematode swimming (thrashing) without morphometry. *BMC neuroscience*, 10(1), 1-6.

Burden, N., Sewell, F., & Chapman, K. (2015). Testing chemical safety: what is needed to ensure the widespread application of non-animal approaches?. *PLoS Biol*, 13(5), e1002156.

Culetto, E., & Sattelle, D. B. (2000). A role for *Caenorhabditis elegans* in understanding the function and interactions of human disease genes. *Human molecular genetics*, 9(6), 869-877.

Elkabti, A. B., Issi, L., & Rao, R. P. (2018). *Caenorhabditis elegans* as a model host to monitor the *Candida* infection processes. *Journal of Fungi*, 4(4), 123.

Gjorgjieva, J., Biron, D., & Haspel, G. (2014). Neurobiology of *Caenorhabditis elegans* locomotion: where do we stand?. *Bioscience*, 64(6), 476-486.

Hale, R., Piggott, J. J., & Swearer, S. E. (2017). Describing and understanding behavioral responses to multiple stressors and multiple stimuli. *Ecology and evolution*, 7(1), 38-47.

Hart, A.C. (2006). Behavior. In: The *C. elegans* Research Community (eds) *WormBook*. doi/10.1895/wormbook.1.87.1.

National statistics, Home Office, ASPA (2020). Annual Statistics of Scientific Procedures on Living Animals, Great Britain

Satapathy, P., Salim, C., Naidu, M. M., & Rajini, P. S. (2016). Attenuation of dopaminergic neuronal dysfunction in *Caenorhabditis elegans* by Hydrophilic Form of Curcumin. *Neurochem Neuropharm Open Access*, 2(111), 2.

<https://www.gov.uk/government/statistics/statistics-of-scientific-procedures-on-living-animals-great-britain-2019>https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/901224/annual-statistics-scientific-procedures-living-animals-2019.pdf

Huang, K. M., Cosman, P., & Schafer, W. R. (2006). Machine vision based detection of omega bends and reversals in *C. elegans*. *Journal of neuroscience methods*, 158(2), 323-336.

Hunt, P. R., Olejnik, N., Bailey, K. D., Vaught, C. A., & Sprando, R. L. (2018). *C. elegans* development and activity test detects mammalian developmental neurotoxins. *Food and Chemical Toxicology*, 121, 583-592.

Hunt, P. R. (2017). The *C. elegans* model in toxicity testing. *Journal of Applied Toxicology*, 37(1), 50-59.

Jadhav, K. B., & Rajini, P. S. (2009). Neurophysiological alterations in *Caenorhabditis elegans* exposed to dichlorvos, an organophosphorus insecticide. *Pesticide biochemistry and physiology*, 94(2-3), 79-85.

Jadhav, K. B., & Rajini, P. S. (2009). Evaluation of sublethal effects of dichlorvos upon *Caenorhabditis elegans* based on a set of end points of toxicity. *Journal of Biochemical and molecular Toxicology*, 23(1), 9-17.

Kaletta, T., & Hengartner, M. O. (2006). Finding function in novel targets: *C. elegans* as a model organism. *Nature reviews Drug discovery*, 5(5), 387-399.

Keller, J., Borzekowski, A., Haase, H., Menzel, R., Rueß, L., & Koch, M. (2018). Toxicity assay for citrinin, zearalenone and zearalenone-14-sulfate using the nematode *Caenorhabditis elegans* as model organism. *Toxins*, 10(7), 284.

Kong, C., Yehye, W. A., Abd Rahman, N., Tan, M. W., & Nathan, S. (2014). Discovery of potential anti-infectives against *Staphylococcus aureus* using a *Caenorhabditis elegans* infection model. *BMC complementary and alternative medicine*, 14(1), 1-17.

Kudelska, M. M., Holden-Dye, L., O'Connor, V., & Doyle, D. A. (2017). Concentration-dependent effects of acute and chronic neonicotinoid exposure on the behaviour and development of the nematode *Caenorhabditis elegans*. *Pest management science*, 73(7), 1345-1351.

Lu, Z., & Qiu, Z. (2017). High glucose concentration restricts fat consumption in *Caenorhabditis elegans*. *INTERNATIONAL JOURNAL OF CLINICAL AND EXPERIMENTAL MEDICINE*, 10(7), 10554-10559.

Maulik, M., Mitra, S., Bult-Ito, A., Taylor, B. E., & Vayndorf, E. M. (2017). Behavioral phenotyping and pathological indicators of Parkinson's disease in *C. elegans* models. *Frontiers in genetics*, 8, 77.

Marsh, E. K., & May, R. C. (2012). *Caenorhabditis elegans*, a model organism for investigating immunity. *Applied and environmental microbiology*, 78(7), 2075-2081.

Mishra, S., Gaur, A. V., & Agarwal, R. (2020). Standardization of synchronization procedure to collect the similar aged *C. elegans*. *Peer Rev. J. Forens. Gen. Sci*, 4, 268-271.

Mishra S., & Agarwal R. 2020. Assessment of behavioural toxicity in dichlorvos-exposed *Caenorhabditis elegans*. *Environmental and Experimental Biology*, 18:305–311.

Raizen, D., Song, B., Trojanowski, N., & You, Y. (2012). Methods for measuring pharyngeal behaviors. In: The *C. elegans* Research Community (eds) *WormBook*. *WormBook*, doi/10.1895/wormbook.1.154.1, <http://www.wormbook.org>

Ruszkiewicz, J. A., Pinkas, A., Miah, M. R., Weitz, R. L., Lawes, M. J., Akinyemi, A. J., ... & Aschner, M. (2018). *C. elegans* as a model in developmental neurotoxicology. *Toxicology and applied pharmacology*, 354, 126-135.

Salim, C., & Rajini, P. S. (2014). Glucose feeding during development aggravates the toxicity of the organophosphorus insecticide Monocrotophos in the nematode, *Caenorhabditis elegans*. *Physiology & behavior*, 131, 142-148.

Salim, C., Thadathil, N., Muralidhara, M., & Rajini, PS (2018). Insights on the age dependent neurodegeneration induced by Monocrotophos, (an organophosphorus insecticide) in *Caenorhabditis elegans* fed high glucose: evidence in wild and transgenic strains. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 211, 15-24.

Stiernagle, T. (2006). Maintenance of *C. elegans*. In: The *C. elegans* Research Community (eds) *WormBook*. doi/10.1895/wormbook.1.101.1, <http://www.wormbook.org>

Tiwari S.S., Tambo F., & Agarwal R. (2020). Assessment of lead toxicity on locomotion and growth in a nematode *Caenorhabditis elegans*. *Journal of Applied and Natural Science*. 12(1), 36-41.

- Trojanowski, N. F., Raizen, D. M., & Fang-yen, C. (2016). Pharyngeal pumping in *Caenorhabditis elegans* depends on tonic and phasic signaling from the nervous system. *Scientific Reports*, 6: 22940.
- Wittkowski, P., Marx-Stoelting, P., Violet, N., Fetz, V., Schwarz, F., Oelgeschlager, M., Schonfelder, G., & Vogl, S. (2019). *Caenorhabditis elegans* As a Promising Alternative Model for Environmental Chemical Mixture Effect Assessment-A Comparative Study. *Environmental science & technology*, 53(21), 12725–12733.
- Xiong, H., Pears, C., & Woollard, A. (2017). An enhanced *C. elegans* based platform for toxicity assessment. *Scientific reports*, 7(1), 9839.
- Zhao, B., Khare, P., Feldman, L., & Dent, J. A. (2003). Reversal frequency in *Caenorhabditis elegans* represents an integrated response to the state of the animal and its environment. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 23(12), 5319–5328.
- Zhen, M., & Samuel, A. D. T. (2015). *C. elegans* locomotion : small circuits, complex functions. *Current Opinion in Neurobiology*, 33, 117–126.

This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).



How to cite this article:

Mishra S., Agarwal R. (2021). An Optimized Assay for Early and Rapid Assessment of Behavioral Alterations using *Caenorhabditis elegans* as an alternate Animal Model *Science Archives*, Vol. 2 (1), 56-61
<http://dx.doi.org/10.47587/SA.2021.2109>