

Elucidation of ageing on low dose exposure to neurotoxicant dichlorvos in invertebrate model *Caenorhabditis elegans*

Abstract

Ageing is a complex process and understanding the factors affecting ageing and end points of study design are complicated and needs to be addressed through various strategies. In this study we have evaluated the ageing process on exposure to low doses of dichlorvos an organophosphorous insecticide used in controlling crop pests and also used in warfare agent and elucidated the various degrees of end points during the life time of an invertebrate model nematode *Caenorhabditis elegans*. This organism dwells in moist soils and it gets exposed to the various toxicants during its life time and this strategy has been modulated in lab conditions by exposing these worms to low doses without affecting the ability of feeding which is essential for survival. The low concentration residues of dichlorvos remain in the water, soil and sprayed areas. The concentrations of dichlorvos 250 and 500nM affected normal feeding as tested by exposing the worms and this induced cessation in feeding at 24h of experimentation. Hence the worms fed at normal rate at 5,100 and 200nM doses were selected for the evaluation of ageing parameters. Synchronized 2d old worm were used for the experiment. Control worms, the AChE activity increased up to 5d and then decreased after 10d. A similar trend was also evident in worms exposed to dichlorvos concentration. The inhibition due to exposure to dichlorvos was evident and maximum inhibition was recorded on day 10. The organophosphorous insecticide inhibits acetylcholinesterase in irreversible manner hence low dosage exposure also inhibited the acetylcholinesterase levels on exposure. The range of inhibition on comparison between 100 and 200nM exposure to dichlorvos in relation to non exposed controls were clearly evident. Similarly, increase in carboxylesterase activity was evident in control worms up to day 5, beyond which the enzyme activity decreased drastically. Quantitation of lipofuscin ageing marker content was increasing with the exposure dose and duration of age and qualitative localization was evident. Our study clearly demonstrates the low dose affect of dichlorvos on ageing process quantitatively and qualitatively in model system.

Keywords: acetylcholinesterase, carboxylesterase, dichlorvos, organophosphates, *caenorhabditis elegans*, ageing, lipofuscin

Volume 2 Issue 5 - 2015

Kisan B,¹ Rajini PS,² Shashikumar Shivaya,² Shruthi NK¹

¹Molecular Biology Unit, University of Agricultural Sciences, India

²Food Protectants and Infestation Control Department, Central Food Technological Research Institute (CSIR lab), India

Correspondence: Kisan B, Molecular Biology Unit, University of Agricultural Sciences, India, Tel 09731938169, Email kisanb1@gmail.com

Received: September 09, 2015 | **Published:** November 20, 2015

Abbreviations: *C. elegans*, *caenorhabditis elegans*; AChE, acetylcholinesterase; CaE, carboxylesterases; DMRT, duncan's multiple regression tests; K-medium, 52mM NaCl and 32mM KCl; FudR, 5-fluoro-2'-deoxyuridine; DTNB, 5, 5-dithio-bis-(2-nitrobenzoic acid)

Introduction

Ageing is an inevitable biological process and has been defined as the progressive accumulation of diverse deleterious changes with time that increases the chance of disease and death. Normal ageing process is characterized by Decline in cholinergic indices (choline acetyltransferase, AChE, and muscarinic acetylcholine receptors).¹

Dichlorvos and organophosphorous compound are widely used for the control of insect/pests and in warfare agent as it inhibits acetylcholinesterase and their by leading to paralysis and death of the exposed organism is well understood. These synthetic compounds reside in the environment and cause serious pollution problems

on low dose exposure. These insecticides exert their main neuro toxicological effects through non-reversible phosphorylation of esterases in the central and peripheral nervous system.² The inhibition of acetylcholinesterase (AChE) and carboxylesterase (CaE) these enzyme systems have been used as a biomarker of organophosphorous insecticide (OPI) exposure.

The model nematode *Caenorhabditis elegans* has been used in understanding in developmental biology, aging, host-pathogen relationship, neuronal diseases and the functions of neuronal circuits (Touch sensation, odour, forward and backward movement). This organism has been widely used because of its simple nervous system, consisting of 302 neurons and its wiring diagram is fully understood and this has facilitated in understanding organism's behavior. Considerable toxicity testing has been performed employing *C. elegans* as a bioassay system and various endpoints have been assessed in both aquatic and soil environments. Most widely used end points include lethality, fecundity and mortality, reproduction, development; growth rate and inhibition of feeding and defecation have also

been extensively evaluated. Ageing is the complex process and *C. elegans* has been used in ageing studies. Many discoveries including dissection of programmed cell death the systematic cloning of the genome the deciphering of the entire DNA sequence (microRNAs, RNA interference and the use of GFP.³

On exposure of *Caenorhabditis elegans* to dichlorvos, worms show a time-dependent response with progressive delays in development, alterations in metabolism, increased cellular repair, and alterations in innate immunity functions. At the latest times, repair processes have to be activated that are presumably a response to damaged nerve and muscles cells.⁴ The changes in gene expression observed in the worms following exposure to dichlorvos pointed towards two potential mechanisms of toxicity: inhibition of AChE and mitochondrial disruption.

Lipofuscin (age pigment) is considered a hallmark of aging since its amount increases with age in most cells.⁵ The rate of lipofuscin accumulation reportedly correlates negatively with longevity (i.e., positively correlates with the rate of aging) several pathological conditions, such as lysosomal storage diseases, radiation injury, tumors are associated with the accumulation of lipofuscin.⁶ Lipofuscin levels have been reported to increase with aging in many organisms, including *C. elegans*.⁷ It has been unequivocally demonstrated that oxidative stress promotes lipofuscin formation. Further, the deposition of lipofuscin in large amounts is likely to make cells considerably more vulnerable to oxidative stress, decrease adaptability and favour the pathologies associated with aging.⁸ The gut granule fluorescence lipofuscin, a complex molecular waste production that accumulates within lysosomes in aging mammalian cells and lipofuscin can contain Schiff bases, which are having similar spectral similarities to the worm blue fluorescence. Consistent with this, blue fluorescence levels increase in aging worm populations.⁹

C. elegans hermaphrodites are demonstrated to display age-related accumulation of fluorescent material characterized by an excitation maximum of about 340nm and an emission maximum of about 430nm. The fact that significant changes in nematode age pigment accumulation can be demonstrated within weeks provides a clear rationale for the use of nematodes to study this phenomenon.

Hence it was of interest to investigate the influence of dichlorvos to affect lipofuscin / aging in the worms especially at concentrations that do not affect the normal physiology and functioning. It was also of interest to follow up the activity of AChE and carboxylesterase (CaE) in the worms as they aged in order to study the impact of dichlorvos on the enzyme activities at different ages.

Our earlier studies demonstrated the potential of dichlorvos to induce oxidative stress and neurophysiological alterations on 4h exposure at 100µm demonstrated the inhibitory effect of dichlorvos on AChE in *C. elegans in vivo*.^{10,11} We have revealed the expression of heat shock proteins expression, decreased brood size, and paralysis and nose contraction on exposure to dichlorvos. The purpose of the present investigation was to study the impact of dichlorvos on the effect on ageing on exposure to dichlorvos by estimating the content of 'age pigment' lipofuscin and effect on acetylcholinesterase and carboxylesterase over the age of the model nematode *C. elegans* by exposing at extremely low concentrations during their life period.

Materials and method

Worm culture and maintenance¹²

Worm culture The nematodes are cultivated on NGM plates (3g/l

1 NaCl, 2.5g/l-1 Proteose Peptone, 5mg/l-1 cholesterol, 1mMol 11 CaCl₂, 1 mmol l-1 MgSO₄, 25 mmol l-1 potassium phosphate, pH 6.0, 17g l-1 agar) on an established lawn of *Escherichia coli* strain OP50 and maintained at 20°C. The worms used for the assays were larvae of (2d) old obtained after a synchronous culture.

Experimental procedure

Feeding: Aliquots (1ml) of K-medium consisting of (52mM NaCl and 32mM KCl) which is routinely used for testing the study compounds with OP50 suspended at a concentration of 1.0 OD (at 550nm) were dispensed into the wells of 12 well plates. Dichlorvos was added to the wells at different concentrations (0, 5, 100, 200, 250 and 500nM). The worms suspended in K-medium were added to the wells such that each well had 5000 worms. The total volume in each well was 1ml. The plate was incubated at 20°C with constant shaking. 1ml sample was drawn at hourly intervals, spun gently for a few seconds at 800rpm and the absorbance of the supernatant was determined at 550nm. The samples were returned to the culture plate for further reading at 12 and 24h. After the exposure period, aliquot of acrylic polymer emulsion (Tristar, Mumbai, India; 20µl of 20mg/ml solution) was added to the worm suspension. The worms were observed after 10min for the pharyngeal localization of the dye.¹³

Worm exposure for acetylcholinesterase, carboxylesterase and lipofuscin: Age synchronized worms (2d old) grown on lawn of *E. coli* grown on nematode growth medium plates obtained and were treated with dichlorvos (0, 5, 100 and 200nM) and grown at low population densities (approx.2000 worms/ml) in K-medium and a relatively constant *E. coli* OP50 concentration of 1.0 O.D at 550nm. 50µM of 5-Fluoro-2'-deoxyuridine (FudR).^{14,15} Age dependent changes in the behavior of *Caenorhabditis elegans* on attraction to *Escherichia coli*.¹⁵ Sterilization and growth inhibition of *Caenorhabditis elegans* by 5-fluorodeoxyuridine.¹⁴ was added this reagent interferes with the egg laying when taken up by the worms during normal feeding and worm's do not develop the progeny. This will facilitate worm number to be constant and age synchronized worms were used for the study. Worms were transferred every day by settling and pelleting and then suspended in fresh medium with dichlorvos concentrations and with 50µM FudR along with OP50 (1 O.D at 550nm). The worms were maintained at 20±1°C and assayed for acetylcholinesterase and carboxylesterase activities at: 0h, 24h, 5d, 10d, 15d and 20d) and lipofuscin was quantified on: 5, 10, 15 and 20d of exposure.

Fluorescence microscopy: On 15d after exposure, worms were immobilized by adding a drop of sodium azide (0.1%) and placed them on slide with cover slip and then observed under phase contrast microscope with a fluorescence module for the localization of lipofuscin under GFP filter and photographed under 10X microscope.⁶

Assay methods

Acetylcholinesterase and carboxylesterase activities: *C. elegans* on treatment with dichlorvos were removed by settling carefully by centrifugation and the dichlorvos treated worms were washed to remove the extra dichlorvos from its body and these worms were thrice washed with K-medium and then the worms were homogenized with 50mM Tris buffer (pH 7.4). The homogenate was centrifuged at 10,000rpm for 10min, and the supernatant (equivalent to 500µg protein) was used for AChE assay. Two hundred micro liter of 1.5mM DTNB and 40µL of 156mM ATCI were added to the supernatant in a 1mL cuvette. The contents were rapidly mixed and the rate of change in absorbance was measured at 10s intervals for 90s in a Shimadzu UV spectrophotometer at 405nm. Kinetic measurements were recorded

and converted to total cholinesterase activity using the extinction coefficient for the colored product, 5-thio-2-nitro-benzoic acid.¹⁶

Carboxylesterase (CaE) (EC 3.1.1.1):¹⁷ Exposed worms were washed with K-medium Homogenate of *C. elegans* was used as enzyme source to determine (15µl) was added to 3ml of Tris-HCl buffer (0.05 M pH- 7.4) and preincubated for 10 min at 30°C in a water bath. The reaction was initiated by adding 50µl of the substrate (20mM PNPA stock solution in acetone) at a final concentration of 0.3mM. The reaction mixture was incubated for 30minutes at 30°C. The absorbance of the reaction product, p-nitrophenol was measured at 405nm against a reagent blank containing PNPA and buffer in a UV-Vis spectrophotometer (Shimadzu). Enzyme activity (CaE-PNPA) was calculated using the extinction co-efficient forp-nitrophenol ($\epsilon_{405}=1.83 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$).¹⁷

Measurement of fluorescent material- lipofuscin:⁷ After exposure, worms were homogenized in methanol (1ml of methanol with 0.5ml of washed nematodes) for 2min on ice. The extract was mixed by vortexing and then centrifuged at 16,000rpm for 10min at 4°C. The fluorescence of the supernatant was measured in a Fluorometer (Shimadzu, Model No: RF-5301PC, Asia specific PTS. Ltd., Singapore) with excitation at 360nm and emission at 415nm.

Protein estimation:¹⁸ 10µl of *C. elegans* homogenate was added to 490µl of distilled water and incubated with 2.5ml alkaline copper sulfate solution for 10min at room temperature 250µl of Folio's reagent (1:1 diluted with water) was added and the colour was read after 30min at 670nm. Bovine serum albumin (BSA) was used as the standard.

Statistical analysis

Data is presented as mean±standard error of three separate experiments performed in triplicate on three different days, using the computer program Excel software (1999). The data were analyzed employing Two way analysis of variance (ANOVA) using SPSS software 10 (Chicago, IL, USA) Duncan's multiple regression test (DMRT) for multiple comparisons was performed to determine the significant differences among the groups. *P*-Values<0.05 were considered significant.

Results

Effect on feeding

Worms were exposed for 12 and 24h to various concentrations of dichlorvos (0, 5, 100, 200, 250 and 500nM) initially to select the dose which had no effect level on feeding. There was a gradual and time-related decrease in bacterial density in the control worm suspension and dichlorvos exposure at 5,100 and 200nM, indicating normal feeding by these worms. This was further confirmed by uptake of red acrylic paint particles which accumulated in the pharynx within 10min. Dichlorvos concentration at 250 and 500nM induced cessation in feeding at 24h of exposure (Figure 1), which was also confirmed by absence of pigment particles in the pharynx clearly indicating cessation in feeding at these concentrations of dichlorvos. Based on the feeding pattern we selected 5, 100 and 200nM of dichlorvos for further studies.

Acetylcholinesterase activity

In untreated worms, the AChE activity increased up to 5d and then decreased after 10d. A similar trend was also evident in worms

exposed to dichlorvos concentration (5, 100nM). However in worms exposed to 200nM dichlorvos, the AChE activity decreased after 5d. As evident from (Figure 2), varying degrees of inhibition in the enzyme activity was evident from 1d in worms exposed to dichlorvos. The inhibition due to exposure to dichlorvos ranged between 12-40% (1d) 18-16% (5d), 29-73% (10d). Maximum inhibition of AChE activity was evident on day 10.

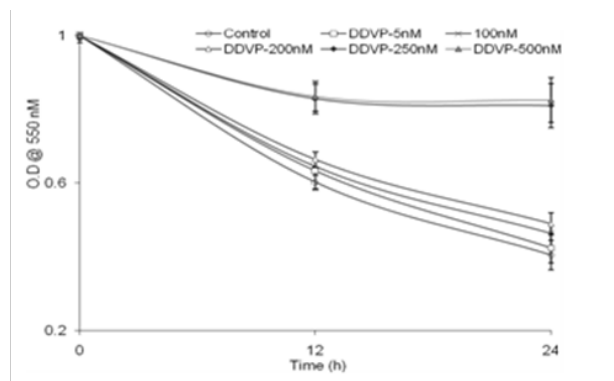


Figure 1 Effect of low concentrations of dichlorvos on feeding in *C. elegans*.

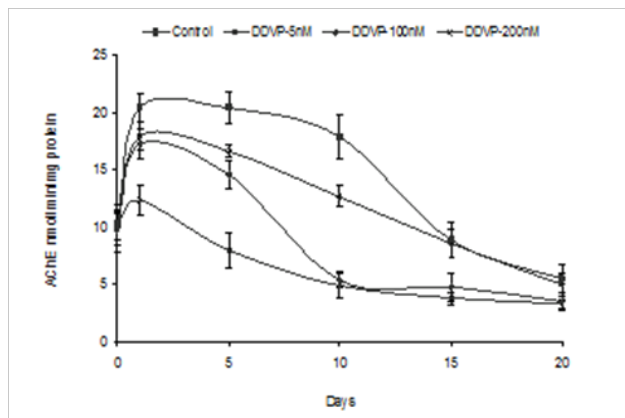


Figure 2 Age-related activity of AChE in *C. elegans*.

Carboxylesterase activity

As evident from (Table 1), and an increase in carboxylesterase activity was evident in untreated worms up to day 5, beyond which the enzyme activity decreased drastically. Interestingly, in worms exposed to dichlorvos, the enzyme activity was significantly higher than control worms on day 1. On day 5-15, the CaE activity in worms exposed to 5 and 100nM was *on par* with that in control worms. The enzyme activity in worms exposed to 200nM was lower than that in control worms at all the time intervals. Interestingly, the enzyme activity in worms exposed to 5nM dichlorvos was higher than control beyond day 5.

Effect on lipofuscin content

Age-dependent increase in lipofuscin content was observed in the control worms. Interestingly, remarkable concentration and age dependent increase in lipofuscin accumulation was observed on exposure to dichlorvos. Dichlorvos at 5,100 and 200nM after 5d of exposure caused 1.3, 4.6 and 5.3 fold increase in lipofuscin levels compared to controls. Similarly lipofuscin accumulation on day 20 at 5,100 and 200nM was 0.6, 1.1 and 1.2 fold higher compared

to controls. While lipofuscin accumulation in control worms was restricted to intestine only, exposure to dichlorvos resulted in significant fluorescence throughout body of the worm (Table 2) (Table 3) (Figure 3).

Table 1 Activity of AChE in *C. elegans* on exposure to dichlorvos with age

AChE (n mol/min/mg protein)						
Dichlorvos (nM)	Day					
	0	1	5	10	15	20
0	10.22a±1.34	20.4b±1.24	20.35c±1.32	17.8c±1.89	8.81c±1.58	5.44c±1.23
5	9.73a±1.45	17.92ab±1.23	16.6±0.56	12.63b±0.89	8.51c±1.23	4.96bc±0.96
100	9.86a±2.05	17.22ab±1.23	14.54b±1.28	5.337a±0.68	4.71b±1.23	3.49ab±0.82
200	10.0a±1.20	12.31a±1.23	7.95a±1.56	4.83a±1.09	3.75a±0.54	3.29a± 0.35

Values are mean±S.E of 3 observations with 3 replicates each.

Data analyzed by post-hoc test (Duncan); Means in the same column with different superscript differ significantly (p<0.05).

Table 2 Activity of Carboxylesterase in *C. elegans* on exposure to dichlorvos with age

Carboxylesterase (nmol/min/mg protein)						
Dichlorvos (nM)	Day					
	0	1	5	10	15	20
0	10.43a±1.23	32.46a±2.03	64.98b±2.56	31.22b±3.62	23.53b±2.78	14.96a±3.45
5	12.00a±3.58	61.55b±10.97	67.72b±4.56	31.95b±2.22	28.78c±2.78	24.00b±1.56
100	12.50a±3.56	63.56b±4.89	63.83b±3.66	12.56a±3.45	17.85a±4.78	13.93a±1.30
200	12.60a±2.67	47.78ab±3.54	36.53a±4.87	13.27a±1.91	15.75a±2.34	13.33a±2.93

Values are mean±S.E of 3 observations with 3 replicates each.

Data analyzed by post-hoc test (Duncan); Means in the same column with different superscript differ significantly (p<0.05).

Table 3 Lipofuscin accumulation in *C. elegans* on exposure to dichlorvos with age

Lipofuscin (Relative fluorescence/mg protein)				
Dichlorvos (nM)	Day			
	5	10	15	20
0	1.89a±0.45	6.29a±0.98	8.38a±1.25	9.10a±1.56
5	4.49b±0.58	10.49b±1.23	13.37b±1.56	14.71b±1.35
100	10.74b±1.20	14.94b±1.76	17.65±1.89	18.68b±1.43
200	12.02c±0.67	16.33c±1.67	18.83±1.89	19.69c±1.45

Values are mean±S.E of 3 observations with 3 replicates each.

Data analyzed by post-hoc test (Duncan); Means in the same column with different superscript differ significantly (p<0.05)

Summary of the results

Worms stop the feeding at the exposure doses of 250 and 500nM. This suggests that the feeding is an important parameter and the dichlorvos affects feeding in the live organism at the exposure dose of 250nM and above hence any organism exposure above certain level of organophosphorous compound is going to stop feeding so as to avoid the consumption or ingestion of the toxicant and so as to protect itself from the accumulation of toxin in the body. This is clearly evident from the model system utilized in this study. The study extended further with low dosage exposure where worms could feed or consume normal feeding has shown that the toxicant accumulated over the period of the exposed time and intern had effect on the

neuronal enzyme acetylcholinesterase by inhibiting the levels ranged from 12-48% from the normal worms. Indicating that the levels below detection by worms could inhibit the acetylcholinesterase and similar observations were recorded for the detoxifying enzyme carboxylesterase. This indicates that the dichlorvos decreases the detoxifying ability of the organism at the lowest exposure. The ageing marker lipofuscin accumulation increased as the worm age increased and the trend was similar on exposed dosages increased with the increased dosage of dichlorvos. The qualitative fluorescence was also observed in intestine. This study indicates that the dichlorvos an organophosphorous compound has profound effect at low dosage and influences on ageing on exposure at low doses.

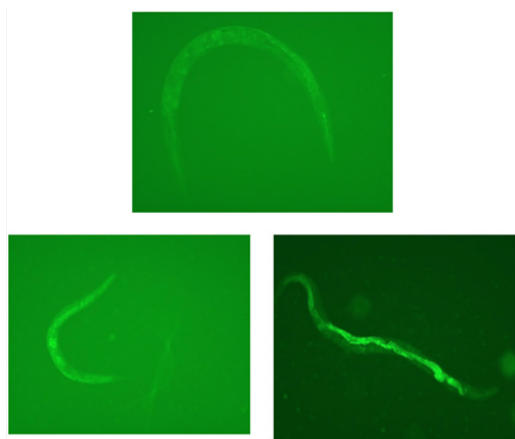


Figure 3 Localization of lipofuscin in *C. elegans* exposed to dichlorvos. A) Control worm B) Worm exposed to dichlorvos (5nM) C) Worm exposed to dichlorvos (200nM) .

Discussion

The soil nematode *C. elegans* has becoming an prominent model organism for understanding aging. The research was focused on genetics of aging and gene mutations leading to increased life span and this led to the popularity of the worm as model system for studying aging and age related disorders. Different approaches are being used in the *C. elegans* aging Viz: Genetic manipulations that influence life span, environmental manipulations such as caloric restriction and hormetic treatments, evolutionary studies, population studies, models of age-related diseases, and drug screening for compounds that extend life span are now being investigated using this nematode.¹⁹

The studies on *C. elegans* have led to the identification of hundreds of genes and regimens that modulate lifespan. Although the initial studies identified genes that altered lifespan and affected dauer diapause, these signalling pathways have nonetheless identified longevity-associated pathways across phylogeny. Aging involves the coordination of multiple systems in an organism and how they change as a function of time.²⁰ Hence in our study we have utilized this model for understanding age related consequences on low dose exposure to dichlorvos an organophosphorous compound which has been routinely used for controlling insect pests worldwide.

In our treatment conditions, we found that feeding was an important parameter to consider for OPI exposure in *C. elegans*. Since there is no literature available on low dose, long-term exposure in invertebrate system, we designed a study based on feeding responses on exposure. We found that dichlorvos induces significant inhibition in feeding beyond 250nM while there were no effects on physiology of worms below this concentration. Based on this criterion, in the present study, dichlorvos concentrations below 250nM and the exposure period of 20d were optimum.

Our results demonstrated that in general the AChE activity increased in worms up to 5d and then decreased after 10d. Maximum inhibition in AChE activity on exposure to dichlorvos was evident at 10d. However, CaE activity increased in untreated worms up to 5d beyond which the activity decreased. Age-dependent increase in lipofuscin content was evident among control worms and interestingly, there was a concentration and age-dependent increase in lipofuscin content was discernible in worms exposed to dichlorvos.¹⁵ Reported that the level of CaEs was significantly different between neonatal

(7days), juvenile (21days), and adult (3months) Sprague-Dawley rats' tissues (lung, plasma, and liver) and showed age dependent increases of CaE activity in all evaluated tissues.

It has also been reported that newborn and young humans also have lower metabolic capacity for some key xenobiotic metabolism pathways compared to adults.^{21,22} This probably accounts for the age-related phenomena observed in animals. In rats, both A and B esterase's increase occurs during the first few weeks postpartum, with a subsequent upward trend to at least 8–9weeks of age.²³ In addition to carboxylesterase, other pathways of organophosphate deactivation exhibit age related increases in activity as the animals mature. Karanth and Pope (2000) reported that the level of CaEs was significantly different between neonatal (7d), juvenile (21d), and adult (3months) rat tissues (lung, plasma, and liver) and showed age-dependent increases of CaE activity in all evaluated tissues. Similarly, age-related increase in brain AChE activity has been demonstrated in both rats and mice.²⁴

The chronological accumulation of age pigment lipofuscin is characteristic of most animals, including humans and the increased accumulation of these substances has been considered the most constant marker of advancing age in animals. These fluorescent compounds include lipofuscin, a heterogeneous mix of oxidized and cross-linked molecules (proteins, lipids and carbohydrates) thought to accumulate in lysosomes.²⁵ It has been suggested that highly cross-linked lipofuscin compounds accumulate in lysosomes where they fail to be efficiently degraded and there by impair normal protein turnover to diminish cell function.⁶ Among humans and small animals, where the increase in lipofuscin accumulation has been extensively studied in heart muscle and nerve cells, it is estimated that 90% of the volume of certain nerve cells in old animals is taken up by age pigment, a fact that indicates that the function of the cell organelles may be impaired.

Studies have also examined age pigment accumulation in senescing.^{26–28} *C. elegans* Age pigment components are known to accumulate at a low rate during adult days (5-10d) in *C. elegans*. That is period as reproductive midlife, but after day 10 (advanced post-reproductive age), the rate of age pigment accumulation increases significantly (day 10 to day 15) which is just after reproduction by self-fertilization normally ceases, suggesting a midlife post-reproductive shift to a distinct physiological state.²⁹ Similarly, we also observed an increased accumulation of age pigment after day 10 in control and significant increase in dichlorvos-exposed worms. This suggests that a metabolic transition, reflected by increased age pigment accumulation, occurs in aging adults.

In the present study, we observed the presence of these granules in the intestine and throughout the body of treated worms, whereas in control worms on day 15, the pigment was visible only in the intestine.

Methyl parathion is reported to cause greater inhibition of brain cholinesterase in neonates compared to adults irrespective of the dosing.^{30–32} Methamidophos was equally inhibitory to brain and blood cholinesterase in neonates and adults following acute dosing.³³ And all cholinesterase endpoints were more sensitive in younger animals compared to adults for methyl parathion. However, younger animals were more sensitive to chlorpyrifos. Similarly, our data on both carboxylesterase and acetylcholinesterase showed greater inhibition in young and adult worms when compared to ageing worms.

Conclusion

In conclusion, our results clearly demonstrate that AChE activity

is maximally inhibited by very low concentrations of dichlorvos after 10d of exposure when the CaE activity is relatively lower. Further, dichlorvos also increased the lipofuscin content in the worms and thereby appeared to accelerate aging in these worms when exposed to very low concentrations.³⁴

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

References

1. Akinduko DS, Adewole TIE. Organophosphate and Ageing Induced Decline in Activity of Acetylcholinesterase Enzyme: Potential Implication on Cognitive Function. *The International Journal of Engineering and Science (IJES)*. 2014;3(1):38–42.
2. Aldridge WN, Reiner E. *Enzyme inhibitors as substrates: Interactions of esterases with esters of organophosphorous and carbonic acids*. 6th ed. North-Holland, Europe; 1972.
3. Tissenbaum HA. Using *C. elegans* for aging research. *Invertebrate Reproduction & Development*. 2015;59(1):59–63.
4. Lewis JA, Gehman EA, Baer CE, et al. Alterations in gene expression in *Caenorhabditis elegans* associated with organophosphate pesticide intoxication and recovery. *BMC Genomics*. 2013;14:291.
5. Terman A, Brunk UT. Lipofuscin: mechanisms of formation and increase with age. *APMIS*. 1998;106(2):265–276.
6. Brunk UT, Terman A. Lipofuscin: mechanisms of age-related accumulation and influence on cell function. *Free Radic Biol Med*. 2002;33(5):611–619.
7. Hosokawa H, Ishii N, Ishida H, et al. Rapid accumulation of fluorescent material with aging in an oxygensensitive mutant mev-1 of *Caenorhabditis elegans*. *Mech Ageing Dev*. 1994;74(3):161–170.
8. Beal MF. Mitochondria, free radicals, and neurodegeneration. *Curr Opin Neurobiol*. 1996;6(5):661–666.
9. Coburn C, Gems D. The mysterious case of the *C. elegans* gut granule: death fluorescence, anthranilic acid and the kynurenine pathway. *Front Genet*. 2013;4:151.
10. Rajini PS, Melstrom PC, Williams PL. A comparative study on the relationship between various toxicological endpoints in *Caenorhabditis elegans* exposed to organophosphorus insecticides. *J Toxicol Environ Health A*. 2008;71(15):1043–1050.
11. Jadhav KB, Rajini PS. Evaluation of sublethal effects of dichlorvos upon *caenorhabditis elegans* based on a set of end points of toxicity. *J Biochem Mol Toxicol*. 2009;23(1):9–17.
12. Jadhav KB, RajiniPS. Neurophysiological alterations in *C. elegans* exposed to dichlorvos, an OPI. *Pesticide Biochemistry and Physiology*. 2009;94:79–85.
13. Brenner S. The genetics of *Caenorhabditis elegans*. *Genetics*. 1974;77(1):71–94.
14. Jones D, Candido EP. Feeding is inhibited by sublethal concentrations of toxicants and by heat stress in the nematode *Caenorhabditis elegans*: relationship to the cellular stress response. *J Exp Zool*. 1999;284(2):147–157.
15. Hosono R. Age dependent changes in the behavior of *Caenorhabditis elegans* on attraction to *Escherichia coli*. *Exp Gerontol*. 1978;13(1–2):31–36.
16. Hosono R. Sterilization and growth inhibition of *Caenorhabditis elegans* by 5-fluorodeoxyuridine. *Exp Gerontol*. 1978;13(5):369–374.
17. Ellman GL, Courtney KD, Andres V Jr, et al. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*. 1961;7:88–95.
18. Sidhu RS, Blair AH. Human liver aldehyde dehydrogenase. Esterase activity. *J Biol Chem*. 1975;250(19):7894–7898.
19. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the folin phenol reagent. *J Biol Chem*. 1951;193(1):265–275.
20. Olsen A, Vantipalli MC, Lithgow GJ. Using *Caenorhabditis elegans* as a Model for Aging and Age-Related Diseases. *Ann N Y Acad of Sci*. 2006;1067:120–128.
21. Tissenbaum HA. Using *C. elegans* for aging research. *Invertebr Reprod Dev*. 2015;59(1):59–63.
22. Johnson TN. The development of drug metabolizing enzymes and their influence on the susceptibility to adverse drug reactions in children. *Toxicology*. 2003;192(1):37–48.
23. Mueller A, Bachi T, Hochli M, et al. Subcellular distribution of S100 proteins in tumor cells and their relocation in response to calcium activation. *Histochem Cell Biol*. 1999;111(6):453–459.
24. Benke GM, Murphy SD. The influence of age on the toxicity and metabolism of methyl parathion and parathion in male and female rats. *Toxicol Appl Pharmacol*. 1975;31(2):254–269.
25. Timchalk C, Poet TS, Kousba AA. Age-dependent pharmacokinetic and pharmacodynamic response in preweanling rats following oral exposure to the organophosphorus insecticide chlorpyrifos. *Toxicology*. 2006;220(1):13–25.
26. Yin D. Biochemical basis of lipofuscin, ceroid, and age pigment like fluorophores. *Free Radic Biol Med*. 1996;21(6):871–888.
27. Klass MR. Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. *Mech Ageing Dev*. 1977;6(6):413–429.
28. Davis BO Jr, Anderson GL, Dusenbery DB. Total luminescence spectroscopy of fluorescence changes during aging in *Caenorhabditis elegans*. *Biochemistry*. 1982;21(17):4089–4095.
29. Braeckman BP, Houthoofd K, De Vreese A, et al. Assaying metabolic activity in ageing *Caenorhabditis elegans*. *Mech Ageing Dev*. 2002;123(2–3):105–119.
30. Gerstbrein B, Stamatas G, Kollias N, et al. *In vivo* spectrofluorimetry reveals endogenous biomarkers that report healthspan and dietary restriction in *Caenorhabditis elegans*. *Ageing Cell*. 2005;4(3):127–137.
31. Pope CN, Chakraborti TK. Dose-related inhibition of brain and plasma cholinesterase in neonatal and adult rats following sublethal organophosphate exposures. *Toxicology*. 1992;73(1):35–43.
32. Liu J, Oliver K, Pope CN. Comparative neurochemical effects of repeated methyl parathion or chlorpyrifos exposures in neonatal and adult rats. *Toxicol Appl Pharmacol*. 1999;158(2):186–196.
33. Pope CN, Liu J. Age-related differences in sensitivity to organophosphorus pesticides. *Environ Toxicol Pharmacol*. 1997;4(3–4):309–314.
34. Moser VC. Comparison of aldicarb and methamidophos neurotoxicity at different ages in the rats: Behavioral and biochemical parameters. *Toxicol Appl Pharmacol*. 1999;157(2):94–106.
35. Olsen A, Vantipalli MC, Lithgow GJ. Using *Caenorhabditis elegans* as a model for aging and age-related diseases. *Ann N Y Acad Sci*. 2006;1067:120–128.