

Comparison of the Toxic Effects of Quinolinic Acid and 3-Nitropropionic Acid in *C. elegans*: Involvement of the SKN-1 Pathway

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Abstract The tryptophan metabolite, quinolinic acid (QUIN), and the mitochondrial toxin 3-nitropropionic acid (3-NP) are two important tools for toxicological research commonly used in neurotoxic models of excitotoxicity, oxidative stress, energy depletion, and neuronal cell death in mammals. However, their toxic properties have yet to be explored in the nematode *Caenorhabditis elegans* (*C. elegans*) for the establishment of novel, simpler, complementary, alternative, and predictive neurotoxic model of mammalian neurotoxicity. In this work, the effects of QUIN (1–100 mM) and 3-NP (1–10 mM) were evaluated on various physiological parameters (survival, locomotion, and longevity) in a wild-type (WT)

strand of *C. elegans* (N2). Their effects were also tested in the VC1772 strain (knock out for the antioxidant SKN-1 pathway) and the VP596 strain (worms with a reporter gene for glutathione S-transferase (GST) transcription) in order to establish the role of the SKN-1 pathway in the mode of action of QUIN and 3-NP. In N2, the higher doses of both toxins decreased survival, though only QUIN altered motor activity. Both toxins also reduced longevity in the VC1772 strain (as compared to N2 strain) and augmented GST transcription in the VP596 strain at the highest doses. The changes induced by both toxins require high doses, and therefore appear moderate when compared with other toxic agents. Nevertheless, the alterations produced by QUIN and 3-NP in *C. elegans* are relevant to mammalian neurotoxicity as they provide novel mechanistic approaches to the assessment of neurotoxic events comprising oxidative stress and excitotoxicity, in the nematode model.

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Introduction

Neuronal cell death caused by degenerative processes, such as those present in Huntington's disease (HD), entails a complex array of toxic events that ultimately lead to brain tissue damage, involving apoptosis, necrosis, or autophagy (Rami 2009). Mitochondrial dysfunction and oxidative stress, two closely related processes, are among the main events linked to neuronal degeneration (Keating 2008). Given the high energetic demands, characteristic of neurons (Bélanger et al. 2011) is not surprising that energy depletion caused by mitochondrial dysfunction represents a major cell death-driving force;

however, mitochondrial defects can also affect cell survival by other means, such as the overproduction of reactive oxygen species (ROS) as a consequence of deficiencies in the oxidative phosphorylation chain (OXPHOS) (Federico et al. 2012). When levels of ROS in cells exceed the capacity of their antioxidant mechanisms, cells are exposed to oxidative stress (Bhattacharyya et al. 2014). Oxidative damage can elicit a variety of harmful effects, including calcium deregulation and damaging of lipids, nucleic acids, and proteins (Federico et al. 2012). Moreover, oxidative stress is not exclusively caused by energetic failure, as it can also originate from other sources, such as excitotoxicity (Pérez-De La Cruz et al. 2012). Indeed, excitotoxicity is currently defined as a toxic event derived from the persistent stimulation of membrane glutamate receptors in neuronal cells (Olney 1990) that is characterized by toxic levels of intracellular Ca^{2+} generated in response to massive opening of Ca^{2+} channels associated to these glutamate receptors (Essa et al. 2013; Mehta et al. 2013). In turn, the toxic cascade elicited by enhanced intracellular Ca^{2+} levels trigger cell damage linked to enhanced reactive oxygen and nitrogen species (ROS/RNS) formation, mitochondrial dysfunction, metabolic deregulation, and further activation of necrotic and apoptotic signals (Rami et al. 1997).

Quinolinic acid (QUIN) is a neuroactive byproduct of the kynurenine pathway, a major tryptophan metabolic pathway that generates NAD^+ as an end product (Pérez-De La Cruz et al. 2012). QUIN acts as a competitive agonist for glutamate-sensitive N-methyl-D-aspartate receptors (NMDAR) on cell membranes (Ting et al. 2009). Under pathological conditions, QUIN levels may be elevated, and when this is the case, this metabolite becomes toxic, causing various noxious effects, including excitotoxicity (and therefore calcium deregulation), overproduction of ROS, mitochondrial energy depletion, and upregulation of NOS activity, to name a few (Guillemin 2012), and these events have served to produce an important phenotypical model of HD.

3-Nitropropionic acid (3-NP) is a neurotoxic compound that has also been used in several models for HD (Borlongan et al. 1997). 3-NP is produced by several species of the genus *Arthrinium* sp. (Alexei et al. 1998), and several plants. This compound exerts its neurotoxic effects through an irreversible inhibition of the respiratory chain complex II (succinate dehydrogenase) that leads to energy deficiency, secondary excitotoxicity, and augmented ROS levels (Silva-Adaya et al. 2008). In addition, it may also disrupt neurotransmitter release (Túnez et al. 2010).

The nematode *Caenorhabditis elegans* (*C. elegans*) has been instrumental for biological research and technology development in different areas (Rodríguez et al. 2013). This soil duelling species is a well-characterized animal model, considering its whole genome has already been sequenced, and vast knowledge on its development and a precise mapping of its nervous system are available (Strange 2006). Its nervous

system comprises cholinergic, GABAergic, glutamatergic, dopaminergic, and serotonergic signalling networks, which have been conserved throughout evolution, thus allowing the worm modelling of mammalian characteristics. For example, *C. elegans* possesses a regulatory antioxidant pathway which is homologous to the mammalian Nrf2 redox modulatory pathway, the SKN-1 pathway (Blackwell et al. 2015). Analogously to Nrf2 in mammalian systems, when transactivated, SKN-1 combats oxidative stress in *C. elegans* (Joshi and Johnson 2012). SKN-1 is known to regulate key phase II detoxification genes by means of constitutive and stress-inducible mechanisms in chemosensory neurons and intestine, respectively. It is also known that SKN-1 mutants are sensitive to oxidative stress and have shortened lifespans (An and Blackwell 2003). Thus, SKN-1 effectively combats resistance to oxidative and xenobiotic stress through the regulation of antioxidant enzymes, such as glutathione S-transferase (GST) and others (An and Blackwell 2003; Martínez-Finley et al. 2013). Key experimental protocols are still needed to support the regulation of the SKN-1 pathway in *C. elegans* as a viable model to mimic different neurodegenerative disorders.

The aims of this work were (1) to evaluate the effects of QUIN and 3-NP on general physiological parameters (survival, behavior, and longevity); (2) to investigate the role of a compromised antioxidant defense system on the susceptibility to these toxins; and (3) to model the activation of antioxidant defenses (GST transcription) against the insults provoked by these substances in the nematode. These approaches were deemed essential to providing basic information useful for further toxicological studies employing *C. elegans* exposed to these toxins as a viable, complementary, and alternative model for neurodegenerative disorders associated with excitotoxicity and oxidative stress. Our results show that both QUIN and 3-NP are capable of altering physiological parameters and induce toxicity in various *C. elegans* strains. Therefore, our data support the concept that these neurotoxic models can be used to reproduce toxic patterns observed in mammals.

Materials and Methods

Reagents and Strains

QUIN, 3-NP (99 and $\geq 97\%$ purity, respectively), and other reagents were obtained from Sigma-Aldrich (St. Louis, MO). Other reagents were purchased from commercial sources. *C. elegans*, N2 wild-type (RRID:WB-STRAIN:RWK40N2) strain and the VC1772 (*skn-1(ok2315)IV/nT1[qIs51](IV;V)*) strain were obtained from the *Caenorhabditis* Genetics Center (CGC, University of Minnesota, Minneapolis, MN). *Escherichia coli* (OP50-uracil auxotroph and NA22 prototroph) was obtained from the Laboratorio de

Bacteriología (Faculty of Medicine, UNAM, Mexico). The transgenic *C. elegans* strain VP596 (dvIs19 III; vsIs33 V), carrying the reporter transgenes Pgst-4::GFP and Pdop-3::RFP, was provided by Prof. Michael Aschner (Albert Einstein College of Medicine, New York, NY).

***C. elegans* Culture and Maintenance**

The three *C. elegans* strains employed herein (N2, VC1772 and VP596) were cultured at 21 °C on a Nematode Growth Medium (NGM) agar plate (containing 0.6 g NaCl, 3.4 g agar (DIBICO, Mexico), 0.5 g Bacto Peptone (Becton Dickinson, Mexico), 0.5 M KH₂PO₄ pH 6, 1 M MgSO₄, 1 M CaCl₂, 5 mg/ml cholesterol, 1 mg/ml Nystatin (Perrigo Lab., Mexico), and 5 mg/ml Streptomycin (PiSA, Mexico)) plus a lawn of the (OP50) strain *E. coli*. The strains were washed with 0.5% saline solution from synchronization agar plate 8P (containing 0.6 g NaCl, 5 g agar, 4 g Bacto Peptone, 0.5 M KH₂PO₄ pH 6, 1 M MgSO₄, 1 M CaCl₂, 5 mg/ml cholesterol, and 1 mg/ml Nystatin) added with a lawn of the *E. coli* (NA22) strain. Next, worms were lysed in 10 M NaOH/hypochlorite solution to obtain eggs.

Survival Test in the Wild-Type (N2) Strain and Strains Treatments

The *C. elegans* strains N2 (wild type), VC1772 (referred to as SKN-1 KO (Martínez-Finley et al. 2013)) and VP596 (dvIs19[pAF15(gst-4::GFP::NLS)];vsIs33[dop-3::RFP], carrying the reporter transgenes Pgst-4::GFP and Pdop-3::RFP (Wang et al. 2013)) were exposed (2000 worms per probe in L1 stage) to increasing doses (1, 10 and 100 mM) of QUIN or 3-NP in acute exposures for 1 h in eppendorf tubes, and then cultured in NGM plates in the presence of *E. coli* (OP50). After 24 h of exposure, 3 full worm cells and 3 empty worm cells were counted, and the average was multiplied by the total number of cells in order to obtain the number of worms in the plate. Eight plates for each treatment were considered for this test and the procedure was repeated independently four times in duplicates.

Locomotion Assessment in the Wild-Type (N2) Strain

Forty-eight hours after exposure to the toxins, locomotion was assessed in worms as the number of “body bends” per worm (Shashikumar et al. 2015). Worms were picked from the NGM-OP50 agar plate and transferred to another NGM plate without bacteria; each worm was allowed to adapt to the environment for 1 min, and then, the number of body bends per 1 min was quantified under the stereomicroscope. A body bend was considered as a change of direction of the cephalic region of a worm, denoted by the presence of the pharyngeal bulb towards the right side. Each body bend was visually

counted as such only when the worm made a whole undulatory movement (Arantes et al. 2016). Eleven worms in each group were tested; tests were repeated independently three times in duplicates.

Longevity Test in Wild-Type (N2) and SKN-1 Knockout (VC1772) Strains

Treated L1 worms from the N2 and VC1772 (KO for SKN-1) strains were grown on NGM-OP50 agar plates and observed daily for survival (until worms die). Worms were transferred to fresh plates daily and incubated at 21 °C. Twenty worms from each group were tested, and the assay was repeated independently in triplicates.

Gst-4 Fluorescence in the GST Fluorescent (VP596) Strain

After exposure to the toxic treatments, the transgenic VP596 strain was washed twice with S-Basal medium and two more times with 0.1% M9-DMSO buffer to keep worms from sticking to the pipette walls, and then transferred to a Costar 96 Bottom Black Side plate to measure fluorescence using a GFP/RFP ratio; worms were paralyzed with 15 µL Levamisole. Fluorescence was quantified with a Cytation3 Multiple Reader (BioTek, VA), and the Gene-5 software, GFP analysis at 485/20 nm and Texas red at 528/20 nm. Images of fluorescent worms were also taken with the Cytation3 Multiple Reader.

Statistical Analysis

All experiments were conducted at least three times. Mean values ± standard error (S.E.M.) were determined for all parameters. Statistical significance among groups was searched by employing one-way analysis of variance followed by post hoc Bonferroni's test.

Results

QUIN and 3-NP Induced Toxicity in the N2 (WT) Strain: Survival and Locomotion Rate

In order to establish the response of worms to both toxins, we first estimated the survival rate in organisms exposed to QUIN or 3-NP in the N2 (WT) strain. Worms exposed either to QUIN or 3-NP showed a significant dose-dependent reduction in survival (Fig. 1). The percentage of survival in controls was taken as 100%. One hundred mM QUIN led to a significant reduction in survival compared to controls ($F_{(3;5)} = 17.41$; $p < 0.05$), whereas 1 and 10 mM QUIN had no significant effects. Ten mM 3-NP induced a significant reduction in survival as compared to controls ($F_{(3;5)} = 17.41$; $p < 0.05$),

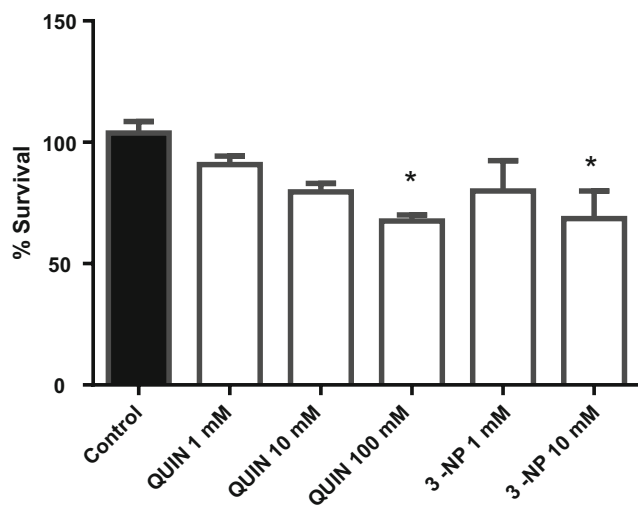


Fig. 1 Effects of QUIN and 3-NP on the survival rate in *C. elegans* (N2). Values represent means \pm S.E.M. ($n = 4$ experiments per group). * $p < 0.05$, different from control; one-way analysis of variance followed by post hoc Bonferroni's test

whereas 1 mM had no effect. One hundred mM 3-NP led to 100% mortality within the 24 h-period of evaluation (data not shown).

Next, the average number of body bends per worm was estimated as an index of the motor/behavioral parameter (Fig. 2). In the control group, worms displayed 28 ± 1.3 body bends per minute. In worms exposed to 1, 10, or 100 mM QUIN, motor activity showed a dose-dependent effect (30 ± 1.7 , 32 ± 1.3 , and 38 ± 0.6 , respectively); only worms exposed to 100 mM QUIN exhibited a significant increase as compared to controls ($F_{(10;5)} = 31.58$; $p < 0.05$). Worms treated with 1 and 10 mM 3-NP did not show any significant behavioral effect as compared to controls (26 ± 1.4 and 26 ± 1.0 , respectively).

QUIN and 3-NP Decreased Longevity in the VC1772 Strain as Compared to N2 Strain

To investigate the involvement of the SKN-1 pathway in longevity of *C. elegans* worms exposed to QUIN or 3-NP, two different strains were compared: N2 (WT) and VC1772 (KO for SKN-1) (Fig. 3). The time course of longevity in these strains, either in the presence or the absence of toxins, is represented in Figure 3A1 and A2. It can be observed that no significant differences were noted in response to treatments when compared with controls. To compare in a clearer manner how the SKN-1 mutation affected longevity, each treatment was graphically dissected out (3B1–3B6). Longevity was not affected by SKN-1 mutation in control worms at any time point (3B1). In contrast, longevity was decreased by QUIN (1 and 100 mM) and 3-NP (10 mM) in the VC1772 strain as compared to the N2 strain during days 5 and 10 of testing (3B2, 3B4, and 3B6; $F_{(19;5)} = 16.65$; $p < 0.05$; significance

not graphically represented for aesthetical reasons), suggesting that the VC1772 strain was more susceptible to the toxins vs. the N2 strain.

QUIN and 3-NP Enhanced the Gst-4 Fluorescence in the VP596 Strain

VP596 strain worms containing GFP as a reporter for glutathione S-transferase (GST) transcription, as well as RFP as a reporter for *dop-3* (as a functional marker of worms' viability), were exposed to increased concentrations of QUIN or 3-NP (Fig. 4). The fluorescence in controls (4A) and 1 mM QUIN (4B) treated animals was significantly lower than in 10 and 100 mM QUIN as well as 1 and 10 mM 3-NP-treated worms, suggesting that GST transcription is linked to SKN-1 gene in these conditions. All images are shown as $\times 20$ magnifications.

Discussion

In this work, we exposed *C. elegans* to two well-known neurotoxins, QUIN and 3-NP, in order to characterize their effects on diverse physiological parameters. The results show that both QUIN and 3-NP exerted toxic effects on various endpoints previously evaluated as predictive of neurotoxicity for worms (Martinez-Finley et al. 2013).

QUIN is an endogenous metabolite from the kynurenine pathway possessing neuroactive properties; however, at nanomolar concentrations, it acts as a selective agonist for NMDAR and as excitotoxin in mammals (Pérez-Severiano et al. 2004). The excitotoxicity produced by the sustained activation of the NMDAR by QUIN has been associated with an increase in cytoplasmic Ca^{2+} levels, ATP depletion, GABA

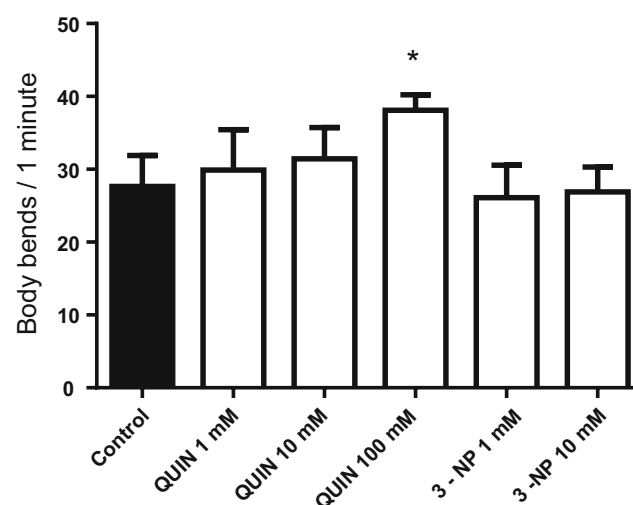


Fig. 2 Effects of QUIN and 3-NP on the locomotion rate (body bends) in *C. elegans* (N2). Values represent means \pm S.E.M. ($n = 11$ experiments per group). * $p < 0.05$, different from control; one-way analysis of variance followed by post hoc Bonferroni's test

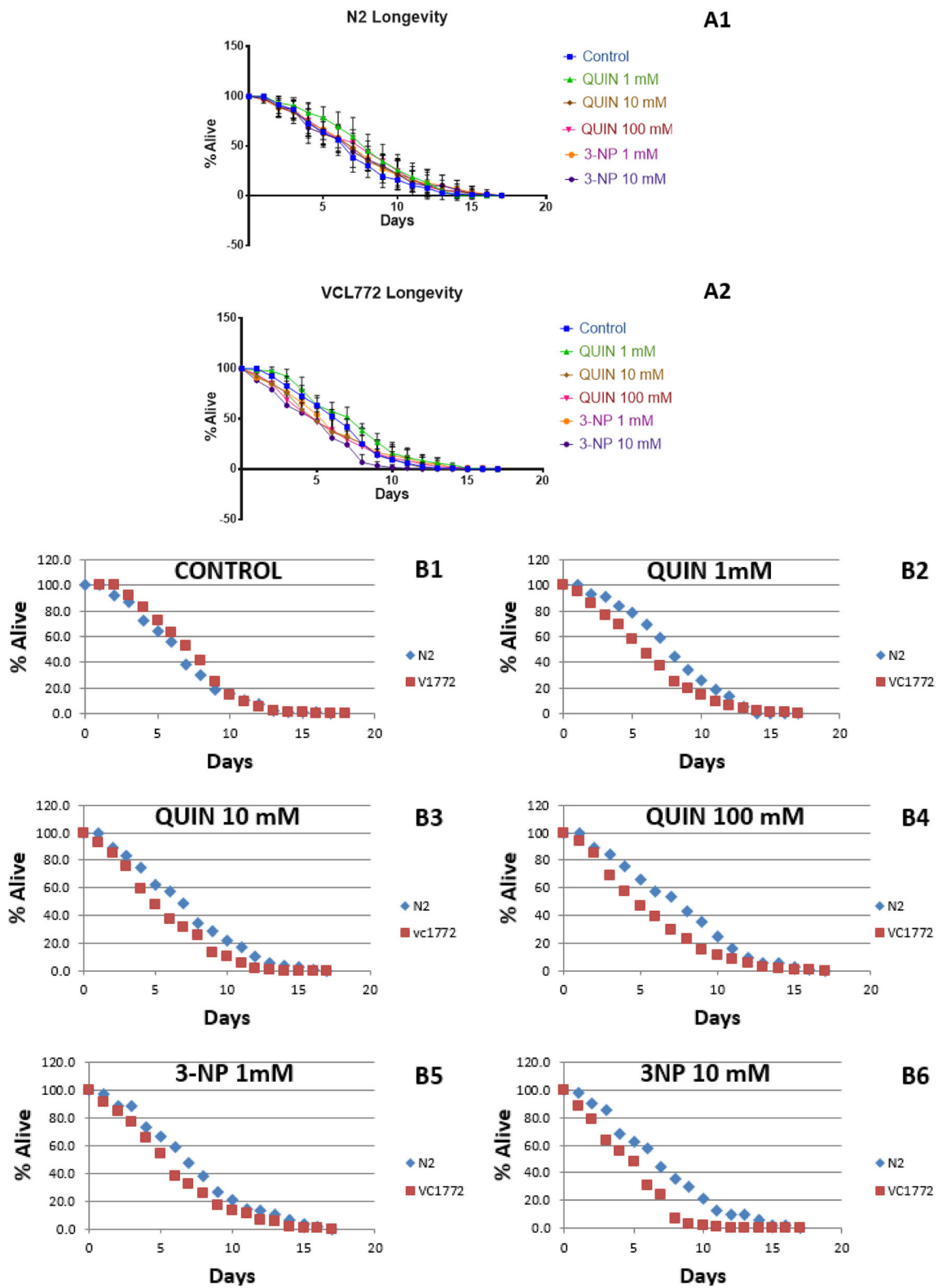


Fig. 3 Effects of QUIN and 3-NP on longevity in N2 (wild type) and VC1772 (KO for SKN-1 gene) *C. elegans* strains. In 3A1 and 3A2, the longevity rate of all experimental groups from the N2 strain is visually compared with that of the VC1772, respectively. In 3B1 to 3B6, the longevity of each experimental condition is visually compared between

the N2 and VC1772 strains (a different and more detailed representation of A1 and A2). Each point represents the mean value \pm S.E.M. of $n = 20$ experiments per group per time point. * $p < 0.05$, different from control; one-way analysis of variance followed by post hoc Bonferroni's test. Statistical significance not graphically represented for aesthetical reasons

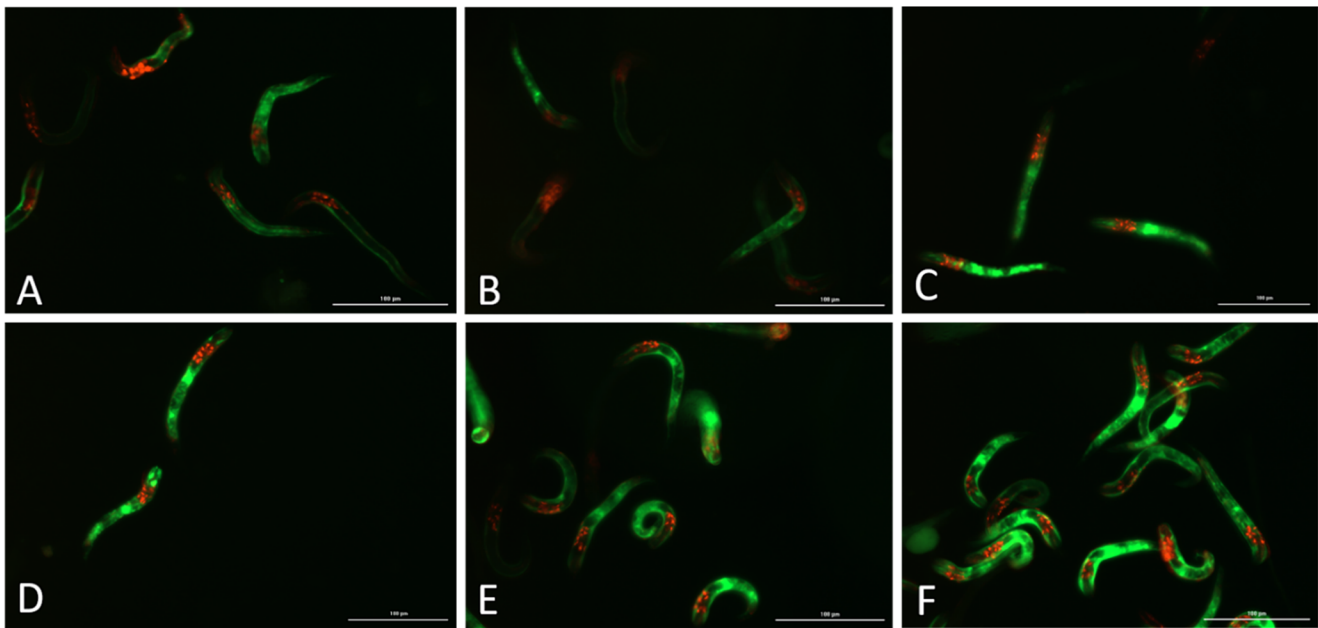


Fig. 4 Effects of QUIN and 3-NP on the *gst-4* fluorescence in *C. elegans* (VP596 strain). Seven experiments were carried out per group. Panels represent *gst-4* expression (in green) in **a** control, **b** 1 mM QUIN, **c**

10 mM QUIN, **d** 100 mM QUIN, **e** 1 mM 3-NP, and **f** 10 mM 3-NP. All images correspond to $\times 20$ magnification

decrease and cholinergic neuronal degeneration, as well as oxidative damage to different cells (Pérez-De La Cruz et al. 2012). Thus, QUIN toxicity arises from its capacity to induce excitotoxicity characterized by increased calcium permeability, decreased ATP formation, oxidative/nitrosative stress, and an ensuing cell death (Pérez-De La Cruz et al. 2012), and this is relevant to the present study as the nematode exhibits functional NMDAR (Kano et al. 2008). In addition, given the homology that SKN-1 and Nrf2 pathways exhibit in *C. elegans* and mammals, respectively, the nematode becomes a useful model to explore these redox regulatory signals as targets in the toxic model produced by QUIN.

In contrast, 3-NP forms a covalent bond with mitochondrial complex II (succinate dehydrogenase), therefore inhibiting its function. The main consequences of this inhibition are an energetic deficit caused by decreased ATP production and a concomitant increase in ROS generation, propagating oxidative stress (Túnez et al. 2004). It has been demonstrated that these phenomena can induce negative consequences on different physiological parameters of *C. elegans*, as previously demonstrated when exposing worms to pro-oxidant agents such as H_2O_2 (Murakami and Murakami 2005; Kumsta et al. 2011). These alterations might be linked to the inhibition of ATP-dependent proteins and their transient deactivation (Kumsta et al. 2011).

Our results support the aforementioned effects, and suggest that both QUIN and 3-NP may play a role as ROS production promoters, as well as inhibitors of ATP synthesis, a mechanism that might partially explain changes in the survival rate and behavior of worms in response to QUIN and 3-NP

exposure. It is also possible that reduction in dopamine synthesis related with oxidative stress might contribute to toxicity and behavioral alterations, given the role of this neurotransmitter in motor regulation in *C. elegans* (Rodríguez et al. 2013). Overall survival was decreased by both toxins at the highest doses, supporting their toxic potency, and validating them as potential tools for the assessment of physiological perturbations in *C. elegans*. It is noteworthy that even at the highest applied QUIN dose (100 mM), survival approximated 50%, whereas 3-NP showed a higher lethality since it produced a similar effect at a lower dose (10 mM), suggesting that differential toxic mechanisms are inherent to these two agents. The apparent low lethality associated with QUIN treatment might be related to the low density of NMDAR present at the *C. elegans* nervous system, given that these receptors are found only in small groups of neurons that control motor (Maricq et al. 2000) and memory functions (Kano et al. 2008). Therefore, damage might not be as extensive as in other organisms that have a greater density of these receptors. Moreover, the two agents tested herein appear to be less toxic to worms than others, such as methylmercury (Martínez-Finley et al. 2013), as evidenced by the lack of effect of these two agents on the longevity rate. Moreover, it is known that the response of *C. elegans* to neurotoxic agents is generally achieved at high concentrations (Martínez-Finley et al. 2013). This also applies for QUIN and NP, which also required high doses to elicit toxicity vs. mammals (Pérez-Severiano et al. 2004; Túnez et al. 2010), where lower doses were effective. Likely explanation for this effect might be related with changes in the worms' feeding behavior to avoid the intake of the

toxins, as it has been already described for *C. elegans* (Xu and Deng 2013). The potential role of avoiding the ingestion of these toxins in worms requires additional studies. Furthermore, the worms possess a thick cuticle; thus in addition to poor ingestion, the high doses required for eliciting toxicity may reflect the low absorption/penetration of the toxins via the cuticle. It is noteworthy that this is a common phenomenon in worm research and has been previously corroborated in studies with other toxins such as MPTP, 6-hydroxydopamine, and other compounds (Ali and Rajini 2012; González-Hunt et al. 2014; Stefanello et al. 2015). Low toxicity has also been described in protocols exposing the worms to metals such as manganese (Avila et al. 2016) and iron (Fagundez et al. 2015). Nonetheless, both toxins were able to exert toxicity in the nematode, therefore supporting their use as toxic models with mechanistic homology to those observed in mammals.

Despite no major differences were noted in basal longevity among WT (N2) and SKN-1 KO strains, the sequelae of QUIN and 3-NP treatments support a protective role for this pathway in their toxicity. This consideration is reinforced by the effects of the same doses of QUIN and 3-NP on GST transcription evidenced by the degree of fluorescence in the VP596 strain, corroborating that SKN-1 might activate antioxidant responses to compensate for oxidative toxicity induced by these toxins. This hypothesis shall be explored in further studies. In the interim, in support to the abovementioned suggestion, it is known that QUIN and 3-NP can regulate the homologous Nrf2 pathway in brain tissue preparations of rodents (Colín-González et al. 2014; Silva-

Palacios et al. 2017), thus demonstrating that changes in redox modulatory pathways actively participate in the toxic patterns elicited by these two agents. Of note, whether QUIN and 3-NP regulate the Nrf2 pathway in other biological systems has yet to be determined. Nonetheless, it has been posited that both toxins might possibly activate inner mechanisms of redox dysregulation, leading to a ROS-mediated Nrf2 transactivation as a compensatory response to the ensuing oxidative stress (Colín-González et al. 2014). We therefore hypothesize that similar mechanisms are operative upon QUIN and/or 3-NP vis-à-vis the SKN-1 pathway in *C. elegans*, though this hypothesis will need further experimentation.

An additional parameter evaluated in this study was the locomotion rate, measured by quantification of body bends. It is known that under normal conditions, *C. elegans* modulates its locomotion rate in response to food availability—namely bacteria (Sawin et al. 2000). Behavioral alterations can occur under diverse conditions. Behavioral arousal, for example, may happen when an animal's responsiveness to specific stimuli is altered as a result of a change in its internal state, a change that is often influenced by external factors such as temperature changes, pH alterations, or exposure to toxic agents, among others (Sawin et al., 2000). Our results showed that only QUIN produced changes in locomotion, suggesting that it requires higher concentrations of 3-NP or longer times of exposure to evoke motor alterations. The question as to whether the effect of QUIN on the motor activity of worms is associated with over-stimulation of NMDAR—which in turn regulates important motor functions via modulation of

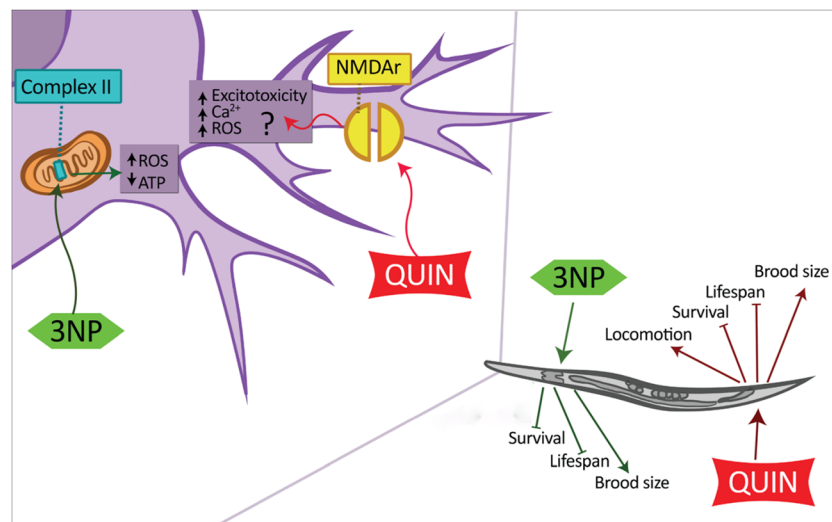


Fig. 5 Schematic representation of the possible mechanisms occurring in the CNS of *C. elegans* strains exposed to QUIN and 3-NP, further leading to alteration of major physiological functions (locomotion, survival, and lifespan). As shown on the left, QUIN might directly act on NMDA subtype of glutamate receptors, thus activating damaging cascades. In

contrast, 3-NP might alter the mitochondrial function, hence recruiting metabolic disruption. In both cases, reactive oxygen species (ROS) formation and oxidative cell damage would further compromise the physiology of the worm (right side)

interneurons (Maricq et al. 2000; Brockie et al. 2001)—remains to be solved. In the interim, we hypothesize that the effects mediated by QUIN involve loss of inhibitory function of these neurons, and probably damage to glutamatergic neurons controlling locomotion, thus precipitating movement dysregulation. Also, to be further explored is the fact that 3-NP was expected to produce more intense effects on motor activity and other endpoints, as this molecule is known to attempt against energy metabolism (Silva-Palacios et al. 2017). Although we do not have an explanation for this, probably the moderate effects of 3-NP found in this study represent effective adaptive responses in the nematode to energy depletion. Nonetheless, the effects of 3-NP in this model need further confirmation under different experimental conditions.

Concluding Remarks

In summary, QUIN and 3-NP were tested in *C. elegans* producing significant alterations in physiological endpoints. The results obtained establish promising findings on the mechanisms of action of QUIN and 3-NP, yet admittedly beyond the characterization provided here, further investigation and detailed analysis are required. A schematic representation of the possible toxic mechanisms driving the effects of these toxins in *C. elegans* is depicted in Fig. 5. Future studies could be profitably directed at the utility of the model in furnishing additional molecular mechanisms of toxicity, and corroborating the utility of this model as a complementary and alternative model to mammalian systems.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

- Alexei T, Hughes PE, Faull RL, Williams CE (1998) 3-Nitropropionic acid's lethal triplet: cooperative pathways of neurodegeneration. *Neuroreport* 9:57–64
- Ali SJ, Rajini PS (2012) Elicitation of dopaminergic features of Parkinson's disease in *C. elegans* by monocrotophos, an organophosphorous insecticide. *CNS Neurol Disord Drug Targets* 11: 993–1000
- An JH, Blackwell TK (2003) SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response. *Genes Develop* 17:1882–1893
- Arantes LP, Peres TV, Chen P, Caito S, Aschner M, Soares FA (2016) Guarana (*Paullinia cupana* Mart.) attenuates methylmercury-induced toxicity in *Caenorhabditis elegans*. *Toxicol Res* 5:1629–1638
- Avila DS, Benedetto A, Au C, Bornhorst J, Aschner M (2016) Involvement of heat shock proteins on Mn-induced toxicity in *Caenorhabditis elegans*. *BMC Pharmacol Toxicol* 17:54
- Bélanger M, Allaman I, Magistretti PJ (2011) Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab* 14: 724–738
- Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE (2014) Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol Rev* 94:329–354
- Blackwell TK, Steinbaugh MJ, Hourihan JM, Ewald CY, Isik M (2015) SKN-1/Nrf, stress responses, and aging in *Caenorhabditis elegans*. *Free Radic Biol Med* 88:290–301
- Borlongan CV, Koutouzis TK, Sanberg PR (1997) 3-Nitropropionic acid animal model and Huntington's disease. *Neurosci Behav Rev* 21: 289–293
- Brockie PJ, Mellem JE, Hills T, Madsen DM, Maricq AV (2001) The *C. elegans* glutamate receptor subunit NMR-1 is required for slow NMDA-activated currents that regulate reversal frequency during locomotion. *Neuron* 31:617–630
- Colín-González AL, Luna-López A, Königsberg M, Ali SF, Pedraza-Chaverri J, Santamaría A (2014) Early modulation of the transcription factor Nrf2 in rodent striatal slices by quinolinic acid, a toxic metabolite of the kynurenine pathway. *Neuroscience* 260:130–139
- Essa MM, Braidy N, Vijayan KR, Subash S, Guillemin GJ (2013) Excitotoxicity in the pathogenesis of autism. *Neurotox Res* 23: 393–400
- Fagundez DA, Câmara DF, Salgueiro WG, NoreMBERG S, Puntel RL, Piccoli JE, Garcia SC, Rocha JBT, Aschner M, Ávila DS (2015) Behavioral and dopaminergic damage induced by acute iron toxicity in *Caenorhabditis elegans*. *Toxicol Res* 4:878–884
- Federico A, Cardaioli E, Da Pozzo P, Formichi P, Gallus GN, Radi E (2012) Mitochondria, oxidative stress and neurodegeneration. *J Neurol Sci* 322:254–262
- González-Hunt CP, Leung MC, Bodhicharla RK, McKeever MG, Arrant AE, Margillo KM, Ryde IT, Cyr DD, Kosmaczewski SG, Hammarlund M, Meyer JN (2014) Exposure to mitochondrial genotoxins and dopaminergic neurodegeneration in *Caenorhabditis elegans*. *PLoS One* 9:e114459
- Guillemin GJ (2012) Quinolinic acid, the inescapable neurotoxin. *FEBS J* 279:1356–1365
- Joshi G, Johnson JA (2012) The Nrf2-ARE pathway: a valuable therapeutic target for the treatment of neurodegenerative diseases. *Recent Pat CNS Drug Discov* 7:218–229
- Kano T, Brockie PJ, Sassa T, Fujimoto H, Kawahara Y, Iino Y, Maricq AV (2008) Memory in *Caenorhabditis elegans* is mediated by NMDA-type ionotropic glutamate receptors. *Curr Biol* 18:1010–1015
- Keating DJ (2008) Mitochondrial dysfunction, oxidative stress, regulation of exocytosis and their relevance to neurodegenerative diseases. *J Neurochem* 104:298–305
- Kumsta C, Thamsen M, Jakob U (2011) Effects of oxidative stress on behaviour, physiology, and the redox thiol proteome of *Caenorhabditis elegans*. *Antioxid Redox Signal* 14:1023–1037
- Maricq AV, Lin C, Brockie PJ, Madsen DM (2000) Genetic analysis of NMDA receptor expression in *C. elegans*. West Coast Worm Meeting
- Martínez-Finley EJ, Caito S, Slaughter JC, Aschner M (2013) The role of SKN-1 in methylmercury-induced latent dopaminergic neurodegeneration. *Neurochem Res* 38:2650–2660
- Mehta A, Prabhakar M, Kumar P, Deshmukh R, Sharma PL (2013) Excitotoxicity: bridge to various triggers in neurodegenerative disorders. *Eur J Pharmacol* 698:6–18
- Murakami S, Murakami H (2005) The effects of aging and oxidative stress on learning behaviour in *C. elegans*. *Neurobiol Aging* 26: 899–905

- Olney JW (1990) Excitotoxicity: an overview. *Can Dis Wkly Rep* 1E:47–58
- Pérez-De La Cruz V, Carrillo-Mora P, Santamaría A (2012) Quinolinic acid, an endogenous molecule combining excitotoxicity, oxidative stress and other toxic mechanisms. *Int J Tryptophan Res* 5:1–8
- Pérez-Severiano F, Rodríguez-Pérez M, Pedraza-Chaverrí J, Maldonado PD, Medina-Campos ON, Ortiz-Plata A, Sánchez-García A, Villeda-Hernández J, Galván-Arzate S, Aguilera P, Santamaría A (2004) S-Allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats. *Neurochem Int* 45:1175–1183
- Rami A (2009) Review: autophagy in neurodegeneration: firefighter and/or incendiary? *Neuropathol Appl Neurobiol* 35:449–461
- Rami A, Ferger D, Kriegstein J (1997) Blockade of calpain proteolytic activity rescues neurons from glutamate excitotoxicity. *Neurosci Res* 27:93–97
- Rodríguez M, Snoek LB, De Bono M, Kammenga JE (2013) Worms under stress: *C. elegans* stress response and its relevance to complex human disease and aging. *Trends Genet* 29:367–374
- Sawin ER, Ranganathan R, Horvitz HR (2000) *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron* 26:619–631
- Shashikumar S, Pradeep H, Chinnu S, Rajini PS, Rajanikant GK (2015) Alpha-linolenic acid suppresses dopaminergic neurodegeneration induced by 6-OHDA in *C. elegans*. *Physiol Behav* 151:563–569
- Silva-Adaya D, Pérez-De La Cruz V, Herrera-Mundo MN, Mendoza-Macedo K, Villeda-Hernández J, Binienda Z, Ali SF, Santamaría A (2008) Excitotoxic damage, disrupted energy metabolism, and oxidative stress in the rat brain: antioxidant and neuroprotective effects of L-carnitine. *J Neurochem* 105:677–689
- Silva-Palacios A, Colín-González AL, López-Cervantes SP, Zazueta C, Luna-López A, Santamaría A, Königsberg M (2017) Tert-butylhydroquinone pre-conditioning exerts dual effects in old female rats exposed to 3-nitropropionic acid. *Redox Biol* 12:610–624
- Stefanello ST, Gubert P, Puntel B, Mizdal CR, de Campos MM, Salman SM, Dornelles L, Avila DS, Aschner M, Soares FA (2015) Protective effects of novel organic selenium compounds against oxidative stress in the nematode *Caenorhabditis elegans*. *Toxicol Rep* 2:961–967
- Strange K (2006) An overview of *C. elegans* biology. *Methods Mol Biol* 351:1–11
- Ting KK, Brew BJ, Guillemin GJ (2009) Effect of quinolinic acid on human astrocytes morphology and functions: implications in Alzheimer's disease. *J Neuroinflammation* 6:36
- Túnez I, Montilla P, Del Carmen-Munoz M, Feijóo M, Salcedo M (2004) Protective effect of melatonin on 3-nitropropionic acid-induced oxidative stress in synaptosomes in an animal model of Huntington's disease. *J Pineal Res* 37:252–256
- Túnez I, Tasset I, Pérez-De La Cruz V, Santamaría A (2010) 3-Nitropropionic acid as a tool to study the mechanisms involved in Huntington's disease: past, present and future. *Molecules* 15:878–916
- Wang R, Mason DE, Choe KP, Lewin AS, Peters EC, Luesch H (2013) *In vitro* and *in vivo* characterization of a tunable dual-reactivity probe of the Nrf2-ARE pathway. *ACS Chem Biol* 8:1764–1774
- Xu JX, Deng X (2013) Biological modeling of complex chemotaxis behaviors for *C. elegans* under speed regulation—a dynamic neural networks approach. *J Comput Neurosci* 35:19–37