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Aging by pollutants: introducing the aging dose $(AD)_{50}$

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Abstract

The global scale of environmental pollution and the accumulation of pollutants in environmental sinks such as soil and surface water sediments call for long-term investigation of relevant target organisms. Consistently, age-resolved toxicology in the nematode roundworm *C. elegans* revealed that effective concentrations of pollutants such as heavy metals and nanomaterials accelerate a variety of age-related phenotypes from reduced locomotion to amyloid protein aggregation and neurodegeneration. We suggest the definition of this acceleration by introduction of the aging dose $(AD)_{50}$ that provides a new metric to characterize adverse effects of pollutants. AD_{50} represents any concentration of a pollutant that significantly accelerates an age-related defect in 50% of the exposed individuals. Comparison of pollutant-exposed with unexposed specimen concerning their age when 50% individuals display a specific aging phenotype indicates the time-frame of this acceleration and defines the corresponding reduction of health span. Application of AD_{50} is invented in the short-lived nematode *C. elegans*, however, provides for a research platform to better understand the role of pollutants in aging across different taxa. Toxicology that addresses the entire life span impacts both, environmental protection of wild fauna as well as health protection in the human population.

Keywords: Aging, *C. elegans*, Environmental pollution, Lowest observed effects level (LOAEL), Mercury, Nematodes, Soil, Sediments, Toxicology, Xenobiotics

Background

Investigation and evaluation of pollutant effects mostly address early development of organisms, while bio-interactions with aging adults receive less attention. However, adverse effects of xenobiotics also concern aging populations, e.g., young, middle-aged and old individuals, which puts the role of environmental pollution in aging into focus. During their life span adults are chronically exposed to subtoxic concentrations of pollutants that exist, persist or accumulate in the environmental compartments air, soil, surface waters and sediments. Acute toxicology is important, however, fails to address adverse effects of omnipresent pollutants on longevity and health span. We, therefore, suggest a novel metric, the aging dose $(AD)_{50}$, to define concentrations of environmental toxicants that accelerate aging, e.g., age-related defects. Such age-related defects include degeneration of functions from the molecular, cellular and organ level that altogether result in reduced fitness and frailty which normally occurs in old individuals, but is prematurely induced by pollutants. When premature aging affects young individuals during or even before their reproduction, survival of the entire population is concerned.

Main text

A combination of worldwide sampling, ultraprecision analytical methods and probabilistic modeling reveals the global scale of current pollutant dispersal. Nanomaterials such as nano-silica are produced in tens of thousands of tons annually and added to a plethora of products including drugs and food. Post-use pollutants such as heavy metals or engineered nanomaterials are distributed globally in the environmental sinks air, surface waters, soil and sediments [8, 19]. Although termed sinks, here, all flora and fauna are exposed to the xenobiotics.

The nematode worm *Caenorhabditis* (*C.*) *elegans* is uniquely suited to learn more about the interactions

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between pollutants and aging. *C. elegans* represents a relevant target organism due to both its dual life as inhabitant of environmental sinks in the wild and tractable surrogate animal model in the laboratory. The short boom-bust life cycle of the worm enables life span-resolved analyses of pollutant effects in an adult organism.

Life span-resolved toxicology in adult hermaphrodite *C. elegans* involves cultivation in 96-well microtiter plates, where each well represents the microenvironment that holds all imaginable options for variation. In the presence of pollutants characteristic aging stigmata can be analyzed in differently aged adult nematodes such as (i) decrease in the rate of locomotion, swimming and pharyngeal pumping, (ii) disorganization of organ morphology (e.g., pharynx, intestine, body wall muscles), (iii) impaired protein homeostasis and increased amyloid formation or (iv) increased neurodegeneration [3, 14, 15, 18]. A representative target of aging is the decline of neuromuscular fitness which can be quantified by plotting the number of worms that show a certain age-related phenotype against the age of the worms in days (Fig. 1a). When they get older adult *C. elegans* swim less and simultaneously show more uncoordinated moves or movement that is restricted to their heads and tails (Additional file 1: Figure S1). Pollutants such as certain nanomaterials accelerate the age-related decline of swimming and other prominent hallmarks of aging (Fig. 1).

Since locomotion fitness decreases in an age-dependent manner within a population of wild-type (N2) *C. elegans*, mock-treated worms represent controls for normal aging (Fig. 1a, blue squares). A comparison between *C. elegans* that are treated with increasing concentrations of nano-silica identifies the concentration that shows an effect, e.g., accelerates an age-related phenotype (Fig. 1a, black squares). We introduce the term aging dose $(AD)_{50}$ for any concentration of a pollutant that significantly accelerates an age-related defect in 50% of the exposed worms (Fig. 1a, dotted lines). The projection of the AD_{50} against the age of pollutant-exposed worms versus that of untreated controls indicates the acceleration of aging by the pollutant in days. This value also demonstrates the reduction of *C. elegans* health span concerning a specific age-associated phenotype.

The concept of AD_{50} allows for identification of toxicants that accelerate aging processes and reduce health span in adult *C. elegans*. It enables the detection of pollutants that turn a young worm into an old worm (Fig. 1b). By age-resolved titration of specific aging stigmata lowest adverse effect level (LOAEL) concentrations and the $AD_{50\text{-}LOAEL}$ can be quantified. In principle AD_{50} is applicable to all species, however, it is more practical in organisms with short life spans such as nematodes, the fruit fly *Drosophila melanogaster* or the planktonic crustacean *Daphnia magna* (Additional file 1: Table S1). These invertebrates are amenable to genetics, high throughput as well as systems biology approaches and thus provide a platform to investigate the role of pollutants in organismal aging processes with respect to molecular signaling pathways and protective anti-aging strategies [4, 9]. Translation of the results obtained in *C. elegans* to other species represents a matter of future investigation. This approach is promising, since depending on the applied bioinformatics, 60–80% *C. elegans* homologues to human genes have been identified [7]. Moreover, certain drug candidates that have been selected in the nematode model organism by high-throughput screening are currently in third level clinical trials.

Notably, AD_{50} intentionally relates to one specific age-related change of phenotype in adult *C. elegans* and does not refer to the entire animal. This is consistent with the idea that, for example, xenobiotic-induced defects of locomotion may, but do not necessarily concur with degeneration of the worms' body wall muscles. Like in the elderly where certain physical disabilities develop independent of cognitive decline and vice versa.

The 'green theory of aging' that suggests the beneficial effects of the upregulation of gene groups related to detoxification and chaperonins on longevity provides a convincing framework [5]. However, it has been shown in *Drosophila* that increased life span and xenobiotic metabolism is not invariably causal [1]. We are just beginning to discover the implications of detoxification pathways for real pollutants, realistic concentrations thereof and pollutant-induced acceleration of organismal aging. To this end more studies are needed that address the chronic, age-resolved effects of pollutants. All of this should be investigated across species to distinguish private from public pollutant-induced acceleration of aging. Recent findings show that already repeated brisk walks along busy roads with respective air pollution have measurable impacts on the respiratory and cardiovascular system of exposed humans [16]. It is conceivable that corresponding long-term investigations reveal early manifestation of age-related defects, e.g., their acceleration by air pollutants. Consistently, it will be important to explore if the AD_{50} serves as a useful tool in human (epidemiologic) studies.

The finding that pollutants accelerate aging in *C. elegans* and other invertebrates has consequences for the field of ecotoxicology that are comprehensively discussed elsewhere [17]. Here one of the main issues concerns the sources that provide for materialization of AD_{50} concentrations in the environment, e.g., the long-term accumulation of contaminants in environmental sinks such as soils and sediments [19]. Also, the consequences of

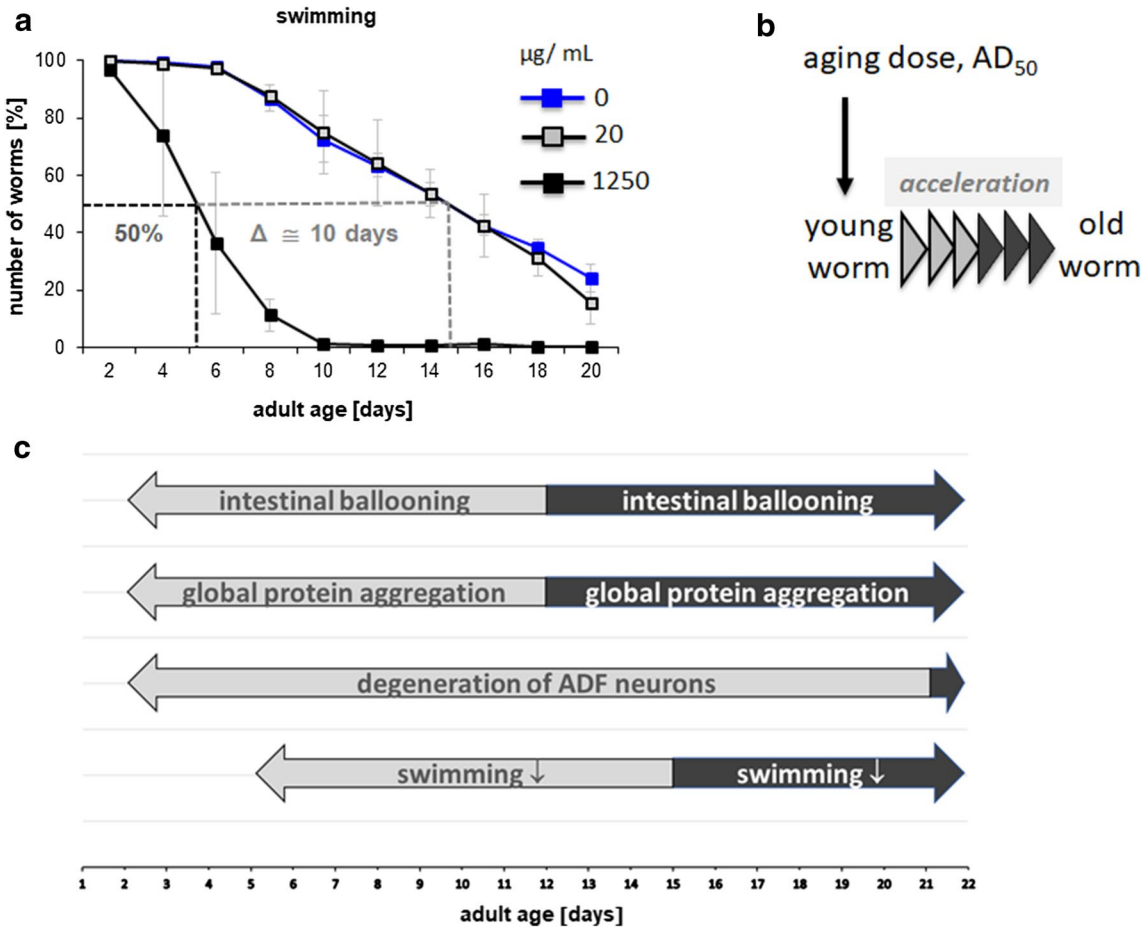

Fig. 1 Aging dose $(AD)_{50}$ demonstrates the acceleration of age-related phenotypes by the representative pollutant nano-silica in adult hermaphrodite *C. elegans*. **a** Populations of adult *C. elegans* were exposed to 0 (blue), 20 (gray) or 1250 (black) µg/mL silica nanoparticles (nano-silica). The locomotion phenotype of swimming was measured over the entire life span of the worms. Adult age is given in days (*x*-axis) and plotted against the number of worms showing the phenotype (*y*-axis). The effect of the pollutant was estimated using two representative concentrations (20 and 1250 µg/mL). Only 1250 µg/mL is effective and thus an aging dose $(AD)_{50}$ that represents the concentration in which 50% of the worms already show a specific age-related defect, while the other half still exhibit the normal phenotype. Notably, exposure to 1250 µg/mL nano-silica accelerates the cessation of swimming in 50% of the worms by 10 days, e.g., from 15-day-old worms to 5-day-old worms (dotted lines). Values represent mean ± SD from three independent experiments with $n = 121–162$ worms per condition per experiment. **b** Schematic: the aging dose $(AD)_{50}$ indicates any concentration of a pollutant that accelerates aging of a young worm concerning an age-related phenotype. **c** Representative for certain other pollutants nano-silica accelerates organismal aging that manifests in biochemical, morphological and neurobehavioral phenotypes. Life span-resolved analyses reveal that hallmarks of aging (black arrows) occur prematurely (light gray arrows), as early as on day 2, in wild-type *C. elegans* exposed to silica nanoparticles [10, 14, 15] (Additional file 1: Table S1)

accelerated aging and reduced health span by pollutants are completely unknown for wild animals. Nevertheless, this question seems topical due to the ongoing biodiversity crisis as pollution-induced premature aging likely adds to the stresses that reduce population fitness in a given habitat. Consistent with this idea it was shown that chronic exposure to field-relevant concentrations of pesticides, e.g., neonicotinoids, induce cognitive defects that impair memory and navigation of bumblebees [13]. Field-relevant concentrations of certain neonicotinoids likewise reduce colony initiation in wild bees and bumblebees which negatively impacts their population dynamics [2, 12].

In *C. elegans* population fitness in response to neurotoxic pollutants is either measured by means of high-throughput platforms [6] or on the level of molecular mechanisms. Certain engineered nanomaterials induce neurotoxicity in *C. elegans* affecting the neuron that controls reproduction. Normally, the serotonergic hermaphrodite-specific neuron (HSN) stimulates egg-laying muscles to contract and eject the worm embryos out of the vulva. Nano-silica enters single cells of the vulva,

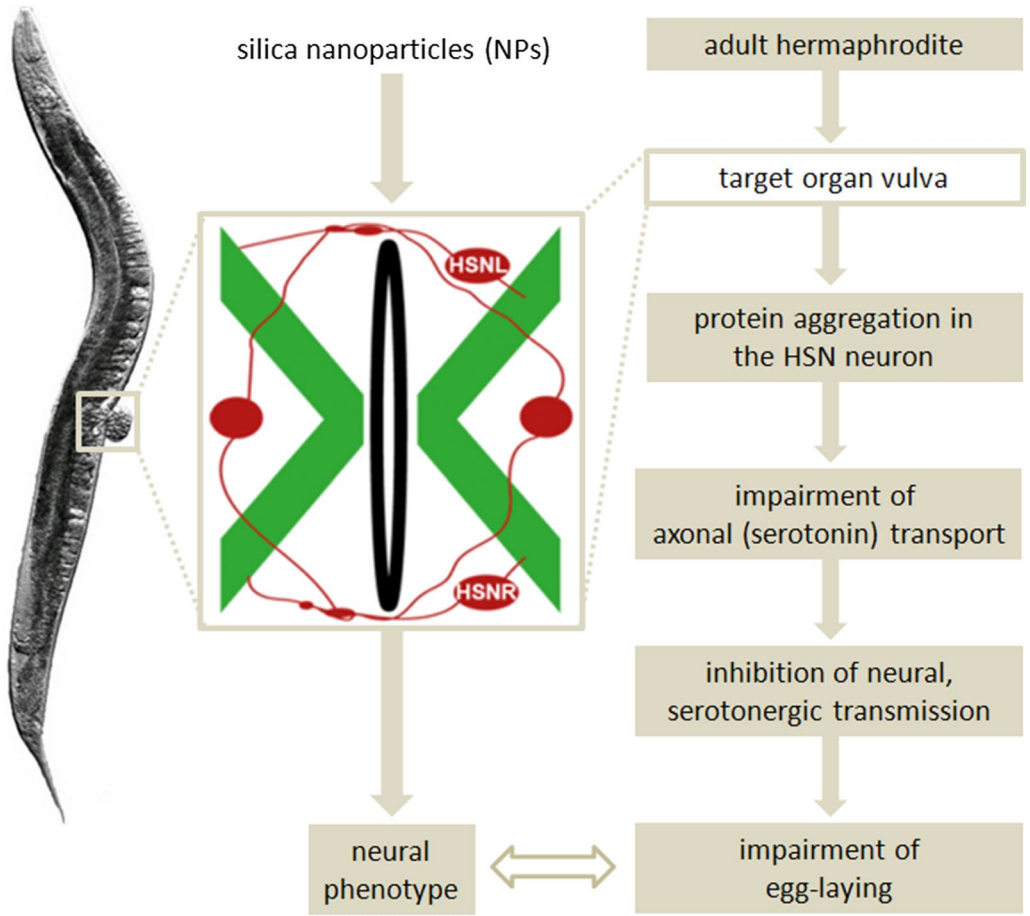

Fig. 2 Schematic depicting a cascade of neurotoxic events that underlie the impairment of reproduction by nanomaterials in the adult hermaphrodite nematode *C. elegans*. HSN, hermaphrodite specific neuron [11, 15]

promotes neurodegeneration of the HSN, disturbs serotonergic signaling in HSN which in turn leads to malfunction of the vulval neuromuscular circuit, a defective vulva and internal hatch [11, 15] (Fig. 2). The example shows that due to exposure with a pollutant, neurodegeneration which normally occurs in old worms is already induced in young *C. elegans* and impairs their reproduction.

Conclusions

Aging dose $(AD)_{50}$ is suggested as a new tool to quantify accelerated aging by pollutants. It facilitates identification of toxicants that promote aging processes and age-related stigmata in adult organisms. Thus, it shifts the focus of toxicological studies from aspects of development to the monitoring of adverse effects in aging adults. While AD_{50} has been established in *C. elegans*, an organism with a short life span, it may likewise be useful in long-lived individuals. Research across species, including humans, is needed to better understand the role of pollutants in aging and age-related diseases. Consistent with this idea the inclusion of AD_{50} into current toxicology and epidemiologic methods represents a promising approach.

Additional file

Additional file 1: Figure S1. Acceleration of age-related locomotory phenotypes by nano silica in aging *Caenorhabditis elegans*. **Table S1.** Acceleration of age-related phenotypes by nanomaterials in *Caenorhabditis elegans, Drosophila melanogaster* and *Daphnia magna*.

Abbreviations

AD_{50}: aging dose; *C. elegans*: *Caenorhabditis elegans*; LOAEL: lowest observed effect level; SD: standard deviation.

Authors' contributions

Conceptualization: AvM; methodology: AP and AvM; investigation: AP; writing: AvM; funding acquisition: AvM; resources: AvM; supervision: AvM. Both authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Not applicable.

Ethics approval and consent to participate
Not applicable.

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