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Review article

Feeding inhibition in daphnids - A sensitive and rapid toxicity endpoint for chemical stress?

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ABSTRACT

The planktonic Crustacea *Daphnia* are among the most employed organisms in ecotoxicology, mainly in regulatory assays that follow OECD/ISO protocols. The most common endpoint for acute testing (24–48 h) without feeding of organisms is usually monitored as mortality or immobilization. A rapid and physiologically and environmentally more relevant toxicity endpoint could be the impaired feeding of daphnids. Decreased feeding of test organisms upon exposure to toxicants has been used to evaluate sub-lethal effects occurring already in minutes to hours. This endpoint, however, has not been used systematically and the respective data are inconsistent due to heterogeneity of experimental design. The aim of this review is to evaluate the scientific literature where impaired *Daphnia* feeding has been used in ecotoxicological research. The search made in WoS (June 5, 2024) using combination of keywords "Daphni* AND feed* yielded 152 articles. Out of these 152 papers 46 addressed feeding of *daphnids* upon exposure to various toxicants (insecticides, heavy metals, pharmaceuticals, contaminated environmental samples and toxic cyanobacteria; in total 59 different chemicals/combinations). These 46 papers formed the basis of the critical analysis presented in the current review. For 18 chemicals it was possible to compare the sensitivity of the feeding and mortality endpoints. We conclude that although the feeding inhibition of *Daphnia* sp. did not prove systematically more sensitive than mortality/ immobilization, it is a sub-lethal endpoint that allows rapid evaluation of toxic effects of chemicals to aquatic crustaceans – important and sensitive organisms in the aquatic food web.

1. Introduction

The planktonic Crustacea *Daphnia* are among the most used test organisms in ecotoxicological research. *Daphnia* have worldwide distribution, albeit mostly in freshwater, and are one of the best studied ecological model organisms to date [1]. *Daphnia pulex* has the first sequenced crustacean genome [2] and the draft genome is also available for *Daphnia magna* [3], the most employed of the *Daphnia* species. Simple culturing in laboratory conditions and physiological characteristics, e.g. short generation time by cyclic parthenogenesis, phenotypic plasticity and body transparency have established *Daphnia* as preferred test organisms with several standardized test guidelines for both acute (ISO 6341:2012 [4], OECD 202 [5]) and chronic (OECD 211 [6]) toxicity by assessing organismal endpoints such as mortality/immobilization and reproductive output.

Beyond the standardized toxicity evaluation, the physiological characteristics of *daphnids* can be successfully applied for the

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development of sublethal endpoints as sensitive early signals of stress, e.g., feeding activity has been proposed as a cost-effective endpoint [7]. Chemicals may reduce the food intake in *Daphnia* but the accompanying signaling pathways are not the same as in case of simply food-deficient environment as shown by Campos et al. [8] who reported upon exposure to toxic chemicals such as heavy metals and insecticides up-regulation of e.g. tissue damage-related genes and importantly, interference with organismal adjustment with limited food intake.

Using feeding inhibition as toxicity endpoint may increase the ecological relevance of the toxicity evaluation and also remarkably reduce the testing time yielding results already in hours [9,10], compared to days and weeks as for immobilization or reproduction assessment. In addition, feeding can be adapted for screening tests in the field [11] mostly for its sensitivity [10]. Feeding response is also relevant for interpretation of other endpoints, such as oxidative biomarkers [12]. However, despite the increasing use of *Daphnia* species in toxicity assessment during last 40 years, the research on the feeding behavior of *daphnids* has been stagnant (Fig. 1).

This review critically analyzed the available data on *Daphnia's* feeding response to chemical stressors with an aim to evaluate the applicability of *Daphnia* feeding inhibition in ecotoxicological research and make suggestions for more consistent use in future studies. Feeding assays using *Daphnia* sp. have the potential to be used as robust yet sensitive tools in chemical hazard assessment.

2. Methodology

Literature search for papers assessing *Daphnia's* feeding under chemical stress was performed in Clarivate Web of Science (June 5, 2024) using combination of keywords "Daphni* AND feed* in the "Title" field. This search yielded 152 articles. This pool of papers was screened and 85 papers were shortlisted for further analysis. Out of these 85 papers 46 contained results of feeding experiments of *daphnids* upon exposure to certain toxicants/chemical stress. The 46 papers form the focus of the current review and are listed in Table S1 and analyzed in the review. Thus, the inclusion criterion was the presence of relevant experimental data in the searched paper. It is important to note that for the purpose of the current review, chemical stress is defined as exposure to xenobiotic chemicals (including excess of essential metals, e.g., copper and zinc), natural and synthetic particulate stressors, cyanobacteria and their toxins and environmental media (contaminated natural waters, wastewater effluents).

In addition to the above described 46 papers, 38 papers out of the 85 were focused on cladoceran feeding ecology (e.g., general feeding behavior and morphology, food selectivity, crowding, maternal effects on feeding) – information that supported the critical analysis of the information on chemical-stress related feeding inhibition.

Data derived from the selected 46 articles concern feeding of *daphnids* upon exposure to certain toxicants and are presented in Table S1. Since one article may have included different test settings, data points rather than articles will be further referred to and discussed henceforth. Altogether Table S1 describes 386 data points 'extracted' from 46 papers. Briefly, one data point is made up of a species/clone of a cladoceran, its age/size class, stressor substance, test design by duration and a feeding endpoint. A more detailed description of how the data point was defined is available in the Supplementary information (SI). In Table S1 the individual data points are characterized by (i) species of Daphnia used; (ii) life-stage of Daphnia used in the test; (iii) description of the chemicals/pollutants such as insecticides, heavy metals, pharmaceuticals or various contaminated environmental samples; (iv) type of algae used as food, algae concentration in the test and methods used for analyses of the food consumption; (v) light and temperature conditions during the test; (vi) duration of the test; test medium.

In addition, curated toxicity data were retrieved from the ECOTOXicology Knowledgebase, U.S. Environmental Protection Agency, http:/www.epa.gov/ecotox/on November 16, 2022. For that all EC_{50} values for the species "*Daphnia*" and feeding related effect measurements were retrieved. The resulting list of chemicals was then searched based on CAS numbers for "*Daphnia*" toxicity values for acute immobilization/mortality effect measurements, see Table S2.

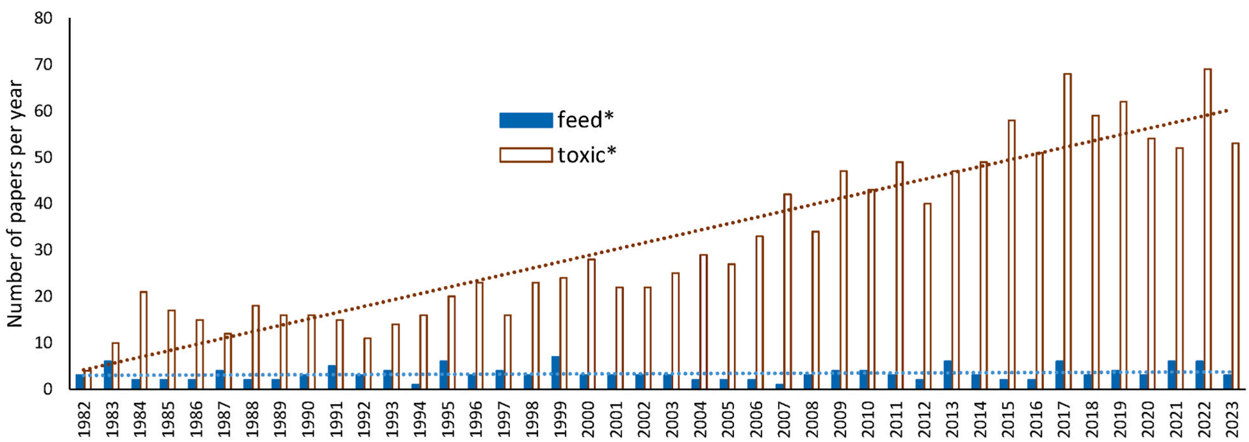

Fig. 1. Timeline (1982–2023) of publications on toxicity research involving *Daphnia* and/or its feeding (Daphni* AND toxic* or Daphni* AND feed* in the "Title" field). Literature search has been performed in Clarivate Web of Science on June 28, 2024.

3. Results and discussion

3.1. Experimental design

Results from experiments on the feeding of *daphnids* can be affected by a number of factors [7] of which selected organismal (age), environmental (temperature, light, feeding medium, stage, duration, sample volume per organism) and food-related (type, concentration of algal cells or particles) ones have been extracted from the reviewed papers (Table S1) and discussed. It is important to note that in all such cases, the assessment of feeding behavior was conducted in conditions to which the organisms had been adapted, i.e. no additional stressor besides the chemical one was applied.

3.1.1. Daphnia characteristics

For pure synthetic chemicals (altogether 146 data points, see Table 3), only 2 different species of *Daphnia* were used in the reviewed feeding studies (Table S1). In line with *Daphnia* research in general [1], *D. magna* was the predominant species being used in 159 data points, whereas *Ceriodaphnia dubia* was only used in 6 data points (Table S1). In literature, for standard endpoints, such as immobilization and reproduction, similar sensitivity was suggested for the two *Daphnia* species across a variety of chemical modes of action [13], however, for feeding inhibition as a toxicity endpoint, more data are needed for comparison.

Daphnia feeding rate depends on their body size, thus data from similarly aged (sized) individuals enables more straightforward comparison. In the feeding studies on pure synthetic chemicals neonatal (<24 h) *daphnids* were used in 35 data points (9 papers), juveniles (1–6 day old) in 48 data points (from 7 papers) and adult (≥7 day old) organisms in 58 data points (from 8 papers). 5 data points from two papers used animals of different ages.

Comparison of the impact of potassium dichromate on neonates and 1, 2, 3-day old juveniles' feeding in parallel showed that neonates and 1-day juveniles were more sensitive than 2-3-day old organisms [14]. For neurotoxic insecticide fenvalerate, it has been shown that even transient feeding inhibition may delay growth but most importantly, maturation [15]. Thus, for the "worst case scenario" as well as a proxy of organismal/population fitness, a feeding assay using neonatal or juvenile *daphnids* may give a more relevant result compared to adult animals.

3.1.2. Test environment

Temperature and light were relatively uniform parameters in the reviewed studies (Table S1). Out of 279 data points where temperature was reported 249 were conducted at optimal temperature, 20-23 °C, in line with ISO6341/OECD202 testing guidelines, i.e. acute test with *Daphnia* sp. One feeding study was carried out at 18 °C (14 data points) and two studies at 25 °C (16 data points). At 25 °C, *Daphnia* filtration and ingestion rates started to decrease significantly from 0.05 mg/L copper sulfate exposure [16] and in 6 h feeding suppression EC_{50} for *Ceriodaphnia* was 0.01 mg/L, which was more sensitive than 48 h immobilization EC_{50}, conducted at 22 °C [10].

Deviations from the optimal, however, have been used to investigate effects of temperature stress. Observations by Lari et al. [17] showed appendage movements and heart rate effectively tripling upon increase of the temperature from 5 °C to 20 °C. Incubation temperatures of 20 °C and 27 °C were used to compare *D. magna* clearance rates of different mixtures of toxic cyanobacteria and green algae (1 data point; [18]), however, the clearance trends were largely similar at both temperatures. From our point of view, feeding test should be performed at 20–22 °C, in this case test results could be compared with standardized endpoints (mortality or reproduction).

Light is another factor which has a significant effect on feeding [19] resulting in highly variable feeding rates [20] and for that, the majority of feeding assessment tests are conducted in the dark, although they are not representative of natural conditions. Light conditions were reported for 237 data points, out of which 155 were carried out in the dark. 58 used constant light or low light while one study (21 data points) [14] used 12 h:12 h light:dark regime and another one [21] used 16 h:8 h light:dark regime (Table S1). To eliminate effect of the light on the feeding rate as well as on the algae growth the feeding test should be preferably performed in the dark.

3.1.3. Test duration, timing

The duration of feeding tests depends on the analyzed feeding endpoint as well as on the assessment method. For instance, short exposure times of up to a few hours have been mostly used to evaluate behavioral endpoints such as limb and mandible movements [22–24] or ingestion rates with radioactive tracers [25–27]. Although the exposure durations ranged from 10 min to 16 days, the vast majority of experiments (357 data points) lasted up to 1 day (Table S1). Disturbances of *Daphnia* feeding due to environmental stressors such as temperature and light fluctuations, presence of suspended solids or cyanobacteria have been reported to occur already within 10 min of exposure [22,28,29]. However, short-term feeding assessments may not have predictive power for long-term effects as feeding rate may increase and decrease within the same feeding assessment as shown at antibiotic azithromycin exposure [30] and for ingestion of heavy metals Cd, Hg, Cr [31]. For heavy metals, recovery of feeding impairment can be due to metallothionein production as a respective detoxification mechanism [31]. Also, chemosensory system-mediated avoidance has been shown to induce rapid recovery of feeding [23,32].

Timing of the feeding is another variable in the feeding assays. Across all the chemical stressors, feeding assessment during chemical exposure ("simultaneous") was largely the parameter of choice (302 data points; Table S1). Post-exposure feeding was studied altogether in 82 data points (Table S1). Regarding water soluble chemicals only, 95 data points were on simultaneous and 56 data points were on post-exposure feeding. Using chemicals with different modes of action, post-exposure feeding has been shown somewhat less sensitive endpoint than simultaneous feeding [33]. However, if using living organisms (e.g. algal, yeast cells) as the food source of *daphnids* in simulatenous feeding setting, potential impact of the studied chemical on the food source itself has to be considered. A parallel sample without the *daphnid(s)* may be appropriate.

3.1.4. Food characteristics

Daphnia are filter feeders grazing on suspended matter in the range of 0,7–70 μm [34]. Concentration of algal cells (food), density of *daphnids* as well as total test vessel volume may affect the feeding rate of *daphnids* [35,36]. In the standardized *Daphnia* reproduction test that lasts 21 days the quantity of supplied algal cells as food should be based on organic carbon (C) content, providing between 0.1 and 0.2 mg C/*Daphnia*/day (in 50–100 ml of medium containing one animal; [6]), that is considered to be the incipient limiting level (ILL) [37]. The ILL is the quantity of food above which the ingestion rate does not depend on food concentration. However, in the papers analyzed for this review, the food quantity was typically reported as cells/ml. In order to compare the studies using various algal species, we converted the cell densities to carbon content (Table S1). Since most groups of phytoplankton, except diatoms, have similar carbon to cell volume relationships, it is possible to use a single C:vol relationship for the 14 different species reported [38]. Cell volumes were retrieved from published datasets [39,40]. We were able to calculate the initial carbon content per animal for 268 data points (out of 330 where algae were used as food). The values ranged from 0.001 to 0.2 mg C/*Daphnia* and most of them were at or below 0.01 mg C/*Daphnia* (162 data points), while the food concentration between 0.1 and 0.2 mg C/*Daphnia* was reported in 31 cases. It should be noted that the ILL depends on animal size, it is several fold lower for neonates compared to adults [41]. In addition to the concentration of food particles in the medium, also the test volume as well as the volume of medium per animal varied among studies. On average there were 9 *daphnids* per sample (1–120, median 5), the sample volume was 122 ml (2–1000 ml, median 100 ml) and consequently the volume per *daphnid* was 19 ml (1–150 ml, median 20 ml). Using food concentrations above the ILL would simplify the interpretation of feeding rate differences among samples, however smaller concentrations may increase the sensitivity of feeding assessment, depending on the assessment method (see paragraph 3.1.5).

To evaluate the feeding behavior in *daphnids*, in addition to living cells, polymeric microparticles may be suitable means. In the shortlisted studies, only Kovács et al., 2012 and Giannouli et al., 2023 applied microparticles (coloured and fluorescent, respectively) as food substitute however the advantages of the concept were already demonstrated decades ago [42]. The major advantage of the use of microparticles in feeding assays is robustness. The results can be obtained fast, already in 30 min [36,43], in miniaturized settings [36] yet the data are in good correlation with the standard 24-h immobilization [42,43] and serve as a sensitive phenotypic endpoint [44]. The major limitation of polymeric microparticles compared to e.g. green microalgae is lack of environmental relevance. Also, although the use of artificial particles creates chemical (microplastic) waste, this could be an acceptable trade-off for resources needed for microalgae handling. The few available literature data are in agreement with microparticles being an effective means for assessing the feeding of *daphnids*.

3.1.5. Feeding evaluation parameters

In evaluation of the feeding activity, filtration, ingestion, digestion and excretion processes could be distinguished. The processes of filtration and ingestion of *Daphnia* involves highly coordinated movement of thoracic limbs thus making them vulnerable to neurological disturbances such as in the case of exposure to neonicotinoid [45], pyrethroid [8,15], organophosphorus insecticides [46] or neurotoxic heavy metals [8,10]. Physical obstructions in the feeding groove can occur because of particulate contaminants such as nanoparticles [47], microplastics [48], suspended solids [49], filamentous algae [50] or cyanobacteria [18,51] impact the collection of food. Compared to ingestion, digestion and excretion have received much less attention as feeding indicators. According to De Coen and Janssen [42], ingestion makes up a much larger share in the organismal energy budget than e.g. digestive enzyme synthesis which makes the former a more important function to adjust in order to cope with toxic stress and hence a more sensitive feeding inhibition

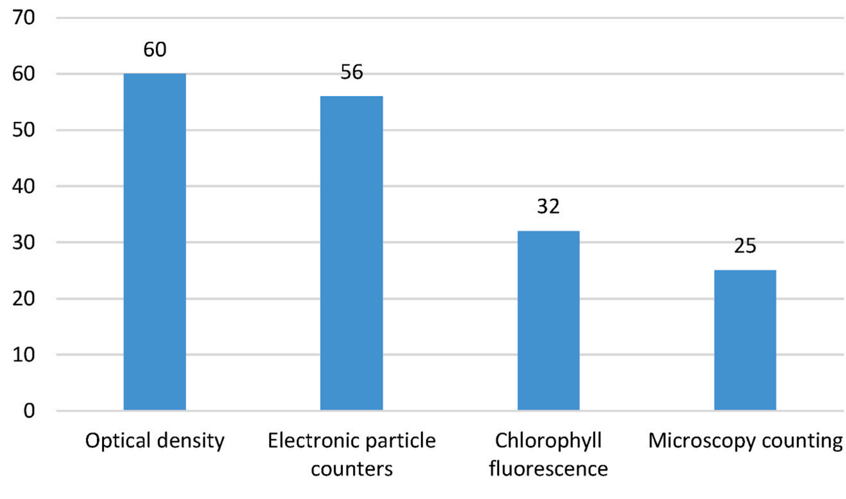

Fig. 2. Food clearance related feeding evaluation methods in the reviewed papers, number of data points. Altogether 173 data points out of 386 contained food source assessment methods (Table S1).

endpoint.

Feeding assessment methods were extracted and grouped by i) analysis of the food source provided to *daphnids* (Fig. 2) or ii) feeding endpoints measured in *Daphnia* (Table 1). One of the simplest methods to estimate feeding activity is to assess the clearance rates of suspended food particles, e.g. green microalgae such as *Chlorella vulgaris* (330 and 91 data points, respectively, Table S1). The main options are recording directly the absolute particle count in suspension or using indirect proxies of cell/particle abundance such as chlorophyll fluorescence or optical density. Counting of cells in test media can be performed with microscopy or electronic particle counters (EPCs; e.g., Coulter counters or flow cytometry; FCM). The clear advantage of FCM compared to just quantifying particles in the feeding medium, is analysis of various parameters simultaneously (particle size and granularity, fluorescence intensity at different emission wavelengths) allowing separation of live algae from partially digested cells and background debris via different fluorescence intensities for each particle respectively [52]. On the other hand, the optical density/fluorescence measurements will include both uneaten and excreted cells, making them less accurate for feeding assessment.

173 data points (45 % of the total 386) contained food source assessment methods with the majority using optical density or cell density by EPCs, but also cell pigment fluorescence measurements or microscopy counting in the feeding medium (Fig. 2). 213 (55 %) data points were on feeding behavior endpoints in *Daphnia*, majority of these being assessed by ingestion and assimilation of radioactively labelled algal cells (111 data points) or visual recordings of feeding physiology (39 data points). Other *Daphnia*-related feeding assessment methods included quantification of food particles/fluorescent microparticles in the gut as well as measurement of digestive enzyme activity (Table 1). In the majority of the reviewed studies, food was provided at 1–2 orders of magnitude lower level than the ILL. In most such studies feeding was assessed by food clearance in the medium where low cell densities may increase the sensitivity of the test, especially when its duration was short and the food was not depleted. In studies where food was provided near the ILL the feeding was generally assessed within *Daphnia* by measuring gut contents, behavior or biochemical markers.

When comparing samples based on *Daphnia* ingestion rate, it is advisable to measure the number of food particles using particle counters/FCM that are relatively easy to use and provide good sensitivity.

3.1.6. Suggestions for feeding test design

In Table 2 we have provided suggestions in order to help reduce the variability in feeding test parameters and facilitate the use of this endpoint in order to better understand the effect of chemicals on *Daphnia* feeding physiology (Table 2).

3.2. Types of chemical stressors investigated in collected publications

In total, the impact of 59 chemical stressors (single chemicals, complex mixtures and toxic cyanobacteria) was studied in the reviewed papers for their impact on *Daphnia* feeding (Table 3; Table S1). The discussion of the current review on the feeding endpoint sensitivity and applicability focuses on water-soluble chemicals that made up 44.6 % of data points of which 108 (28.0 %) were on organic and 68 (17.6 %) on inorganic compounds. Further, due to the scarcity of the available comparative data, in-depth analyses of *Daphnia* feeding endpoint sensitivity was focused on the chemicals with at least 2 data points: one feeding-related and one mortality from within the same study. Such analysis was feasible for 18 chemicals (see “3.3 Sensitivity of feeding as an endpoint”).

3.2.1. Effect of organic chemicals

Of the 28 water soluble organic chemicals, excluding mixtures, 14 were pesticides or their degradation products, followed by pharmaceuticals and flame retardants (Table 3).

Most of the studied pesticides were insecticides that act on the nervous system of target organisms either as acetylcholine receptor

Table 1
Methods for the evaluation of feeding in *daphnids* in the reviewed papers, tabulated as the respective data points. Data are taken from Table S1. See also Fig. 2 for food clearance evaluation methods used.

	Parameter	Method	Data points (total 213)
Ingestion and assimilation	Isotopic tracers	^{14}C activity	101
		^{32}P activity	5
		^{32}P and ^{33}P activity	5
		^{15}N content	1
	Enzyme activity	amylase	8
		trypsin	8
		lipase	2
		β-galactosidase	2
	Gut contents	microscopy counting	24
		microparticle fluorescence	10
		chl a HPPC	5
		x-ray sp.	3
	Gut peristaltics	visual recording	3
	Oesophagus peristaltics	visual recording	2
Feeding behavior	Thoracic limb beat rate	visual recording	11
	Mandible rolling rate	visual recording	11
	Post-abdominal rejection rate	visual recording	7
	Labral rejection rate	visual recording	5

Table 2
Suggestions for designing experiments to evaluate the impact of a chemical toxicant on feeding of *Daphnia* species. The suggestions are based on the analysis of the reviewed literature on *Daphnia* feeding and authors' personal experience.

Daphnia feeding evaluation parameter	Suggestion for feeding experiment	Rationale for suggestion	Comments
Test organism			
Daphnia species	*Daphnia magna*	The most data available for comparison	*C. dubia* was the 2nd most used species of comparable sensitivity to toxicants for standard endpoints (Connors et al., 2022)
Organism life stage (age)	Neonatal (<24 h) or juvenile (1–6 days)	More sensitive than adults	Younger juveniles were more sensitive than older ones (Shashkova et al., 2013)
Exposure conditions			
Temperature	20–23 °C	The most optimal temperature range for *Daphnia*; The most data available for comparison	In line with ISO/OECD testing guidelines
Light	Dark regime	*Daphnia* feeds in a more uniform way. Algal growth is limited, simplifying feeding assessment	
Timing	Simultaneous feeding (feeding assessment during chemical exposure)	Simultaneous feeding has been shown more sensitive than feeding after chemical exposure. The most data available for comparison	Simultaneous feeding was used in most of the reviewed *Daphnia* feeding studies
Duration	Very dependent on method of choice but longer (e.g. 24 h) duration is ecologically more relevant	Longer duration will more likely show potential fluxes in feeding and potential feeding recovery.	Feeding disturbance can occur already within 10 min of exposure but opting for short duration should be method-driven
Medium	Particle-free artificial freshwater	Particles may interfere with filtering, food capture, rejection; induce false satiety and damage digestive tract thus affecting feeding. Via heteroagglomeration with food (e.g. alga cells), particles may also induce its sedimentation Particles may enhance ingestion of positively charged substances	
Food			
Type	Green microalgae		
Concentration	0.1–0.2 mg C/*Daphnia*/day	For constant control (non-exposed) *Daphnia* ingestion, algal concentration should exceed the incipient limiting level (ILL). 0.1–0.2 mg C/*Daphnia*/day is also in line with OECD (2008) testing guidelines. Food concentration should be reported in mg C/*Daphnia*/day to facilitate data comparison.	Incipient limiting level depends on the organism size. For increased endpoint sensitivity, short test duration and/or other feeding endpoints than ingestion, lower than ILL could be considered (majority of reviewed studies)
Feeding evaluation			
Feeding evaluation endpoint	Food clearance from the medium as a proxy of ingestion activity	This is one of the simplest methods to evaluate feeding. Ingestion is a sensitive feeding inhibition endpoint. The most data available for comparison	
Feeding evaluation method	Flow cytometry, electronic particle counter	More informative/accurate compared to measurement of OD/chlorophyll; more cost-effective than microscopy counting	
Positive control chemical	Cd^{2+}	Cd is a non-essential heavy metal and a neurotoxicant that affects *Daphnia* feeding at 0.002 mg/L [53,54] and decreases feeding rate (Campos et al., 2021). The most data available for comparison	In the reviewed papers, Cd and Cu were the most studied chemicals for feeding studies

blockers such as imidacloprid [45], AChE inhibitors like diazinon [46], or by impairing the Na^+/K^+ transport: *cis*-permethrin, [33]; λ-cyhalothrin, [8,33]; cypermethrin [55], whereas endosulfan and lindane impair the GABA-gated chloride channel function [12,46] among other modes of action.

Since feeding in *Daphnia* is primarily a neurologically driven process, i.e., movement of thoracic limb, mandibular, labral and post abdominal muscles, neurotoxicants affect feeding. *Daphnia* will have reduced or less coordinated swimming [55], impaired food collection by thoracic limbs and grinding of mandibles, and there may also be an effect on gut peristaltics [29].

In addition to synthetic chemicals, cyanotoxins microcystin-LR (produced by *Microcystis aeruginosa*) and nodularin (produced by *Nodularia spumigena*) were studied in 30 (7.8 %) data points.

3.2.2. Effect of inorganic chemicals

With the exception of bromine, lithium and aluminum all inorganic water-soluble chemicals in the reviewed articles can be classified as heavy metals of which Cd and Cu were the most studied (5.4 % and 4.1 % of data points, respectively; Table 3). Cd and Cu were shown to affect distinct gut transcriptomic functional signaling pathways: Cd significantly decreased feeding rate whereas Cu

Table 3
The impact of chemical stressors on feeding performance of *daphnids*. Data are summarized from Table S1 and are based on analysis of 46 scientific papers focused on impaired feeding of *Daphnia* upon exposure to different toxicants.

Stressor type			Data points
Water soluble	44.6 %		
Organic single chemicals	22.0 %		
	Pesticides	Cypermethrin	5
		Tetradifon	5
		Fenvalerate	2
		Lindane	2
		λ-cyhalothrin	2
		2,4,6-trichlorophenol	1
		3,4-Dichloroaniline	1
		cis-Permethrin	1
		Diazinon	1
		Endosulfan	1
		Hexachloroethane	1
		Imidacloprid	1
		Pentachlorophenol	1
		Pirimiphos-methyl	1
	Drugs	Azithromycin	8
		Citalopram	1
		Mirtazepine	1
		Haloperidol	1
		Diltiazem hydrochloride	1
		Propranolol hydrochloride	1
		Diclofenac sodium	1
		Metformin	1
		L-Nicotine	1
	Cyanobacterial toxins	Microcystin-LR	18
		Nodularin	12
	Flame retardants	PBDEs	9
	Other	Fluoranthene	4
		DTDMAC	1
Organic chemical mixtures	**6.0 %**		23
Inorganic	**17.6 %**		
	Metals	Cd^{2+}	21
		Cu^{2+}	16
		Cr^{6+} ($K_2Cr_2O_7$)	11
		Ni^{2+}	6
		Zn^{+}	7
		Br^{-}	1
		Pb^{2+}	1
		VO^{3-}	1
		$Al3^{+}$	1
		$Co2^{+}$	1
		Li^{+}	1
		Zr^{4+}	1
Particulate	**7.0 %**		
Suspension	Environmental particles	Clays, silt	13
	Microplastics	Polyethylene	2
		Polystyrene	1
	Synthetic nanoparticles	Polystyrene	1
Emulsion		Paraffin oil	5
		Crude oil	5
Cyanobacteria	**24.4 %**		
		Mircocystis aeruginosa	55
		Anabaena flos-aquae	17
		Aphanizomenon flos-aquae	14
		Raphidiopsis raciborskii	7
		Planktothrix agardhii	1
Environmental samples	**23.1 %**		
Wastewater		Oil sands process-affected water	27
		Industrial	9
		Municipal	2
Natural water		Stream/reservoir water	37
		Cyanobacterial mix	3
		Chaoborus kairomones	8
		P enriched lake seston	3

DTDMAC - dimethyldioctadecylammonium chloride.

induced tissue damage in *Daphnia* gut [8]. Differently from waterborne exposure, foodborne exposure to Cd induced irreversible inhibition of *Daphnia*'s feeding [56] that has been associated with enhanced intake of Cd-ion due to their sorption on algal cells [9].

3.2.3. *Other chemical stressors and cyanobacteria cells*

Particulate matter/chemicals and environmental samples were also addressed in the reviewed articles (Table 3; Table S1). Particulate matter stressors covered 27 (7.0 %) data points and were of natural (clay, silt, crude oil) and synthetic (paraffin oil, polystyrene nano- and microplastics) origin (Table 3; Table S1).

At elevated concentration, particles have the potential to mechanically disturb the feeding of *Daphnia* by e.g. clogging the filtering appendages and food groove, making it difficult to move thoracic limbs for proper aeration and food collection [48], decrease mandibular beat and swallowing while increasing rejection events [22,49]. Similar behavioral responses are characteristic to *Daphnia* while feeding at excess algal density [49]. In addition, particulate matter may induce false satiety [29], especially in case egestion of particles is incomplete as has been shown for polystyrene nanoplastics [57]. Particulate interference may not be confined to physical effects but chemical composition of particles can also have an impact as has been shown for silver [47] and uranium [58] nanoparticles and may cause more damage to *Daphnia* digestive tract than the respective soluble forms [58].

Particles have the potential to affect *Daphnia* feeding also indirectly. Heteroagglomeration with food particles can induce sedimentation of food and even if resuspended, (nano)particle-associated cells would be difficult to digest [59]. By sorbing other chemicals [56,60], particulate matter and algal cells can increase ingestion and effect of positively charged substances [9]. For the purpose of the current review "environmental samples" (89 or 23.1 % of data points; Table 1) were defined as media from contaminated sites (wastewater, oil sands), crude oil, phytoplankton assemblages, chemical cues from predators (*Chaoborus* kairomones; [61]).

Thirteen different strains of toxic and non-toxic cyanobacteria cells were studied in 97 (25.1 %) data points. Cyanobacteria have evolved to produce toxins in order to suppress the feeding of predators such as *D. magna* [28]. As most of the studied insecticides, cyanotoxins are also acting on neurological pathways as shown with ***Planktothrix agardhii*** anatoxin-a [8]. Not only do they elicit neurotoxicity but also cause oxidative stress that can lead to tissue damage in the *Daphnia* gut [8]. For a more complete analysis of interactions between *daphnids* and toxic cyanobacteria the reader is referred to a recently published review [62].

Due to the above described reasons, particulate matter and chemicals of particulate nature as well as the contaminated

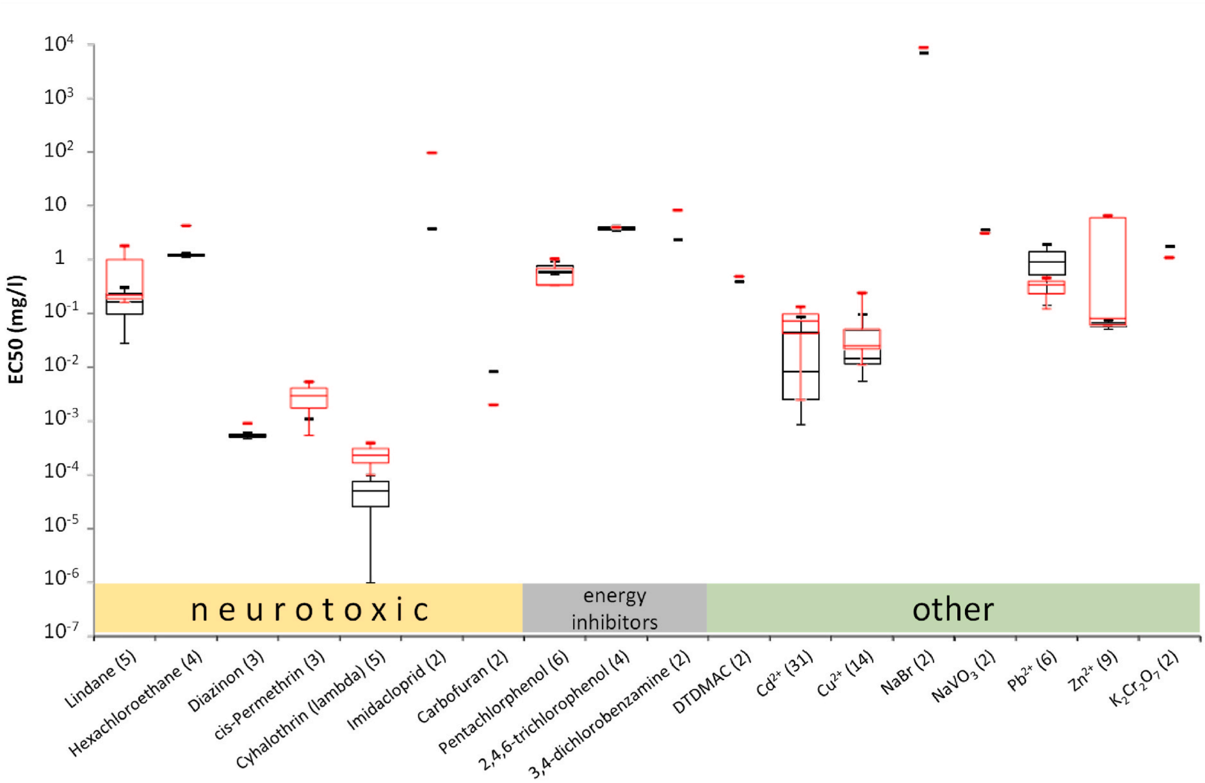

Fig. 3. Comparison of EC_{50} values derived using feeding (black) or mortality (red) as toxicity endpoints. The data have been extracted from the reviewed papers as well as the ECOTOXicology Knowledgebase on the condition that the same studies contained both endpoints (feeding and mortality) for each substance, see Table S2. The numbers in brackets following the chemical names show the sum of data points (feeding plus mortality) per substance; DTDMAC - dimethyldioctadecylammonium chloride. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

environmental samples were not included in the feeding endpoint sensitivity and applicability discussion (see section 3.3).

3.3. Sensitivity of feeding as an endpoint

Impaired feeding has been observed in toxicant concentrations by a magnitude lower than standard endpoints assessed in the same exposure duration [9,30,33,45,63]. In addition, several of the reviewed articles point at shorter exposure time to produce a significant effect on feeding behavior [10,25,35,44,46,64–67]. However, when all available feeding/mortality data (extracted from the selected papers as well as from the ECOTOXicology Knowledgebase) were compared for different chemicals, no clear conclusions about sensitivity could be drawn (data not shown). This was probably due to the wide variation in the reported feeding-related toxicity values that were generated using different experimental settings. Thus, we curated the data by selecting only the feeding and mortality ($EC/IC/LC_{50}$) values that had been reportedly generated within the same studies. This resulted in 18 substances that had at least one EC_{50} value related to both feeding and mortality (Fig. 3, Table S2). Only Cd and Cu had more than 10 such values available.

Despite the rigorous selection the values still come from feeding tests of various durations (1 h–2 days) and mortality tests that typically last 2 days without food. The age of the *daphnids* was the same in individual studies, however, it ranged from neonate to adult across different studies. Shorter exposure time and presence of food is expected to reduce toxicity, however, as a rule, the feeding endpoints were more sensitive than mortality as illustrated by their smaller EC_{50} values on Fig. 3. This is clearly visible for the insecticides that can all be classified as neurotoxic (Table S1). Only in the case of carbofuran is the mortality EC_{50} value smaller than the feeding EC_{50} value which is probably related to the short (1 h) feeding test in this case [68]. Apparently, it takes a longer exposure for carbofuran to affect feeding. It is conceivable that neurotoxic chemicals disturb appendage movement and therefore inhibit feeding activity [46].

For both the energy metabolism inhibitors as well as the "other" chemicals on Fig. 3 the differences in EC_{50} values are small, suggesting the feasibility of feeding endpoints in toxicity assessment. Muscle contraction related to feeding takes up a substantial part of the energy budget of *Daphnia* [42], which explains the effect of oxidative phosphorylation uncouplers (pentachlorphenol, 2,4,6-trichlorophenol) as well as chemicals causing methaemoglobin formation (3,4-dichlorobenzamine) that both inhibit energy production. While sodium bromide can be considered non-toxic, the metals shown on Fig. 3 all either impair Ca and/or Na homeostasis, or, in the case of Cu, catalyze the formation of reactive oxygen species [69,70]. Imbalance of Ca may have neurological consequences since it is required for the transmission of nerve impulses. In addition, Cd affects AChE activity in fish [71] and induces a decrease in physical activity of *Daphnia* consistent with neurological damage [72]. For feeding tests, Cd may be the preferred positive control due to its potency as well as the number of existing data points in the literature.

4. Conclusions

The analysis of the available literature showed that currently in toxicity research involving *Daphnia* species, feeding test is not systematically applied and the existing data are largely inconsistent due to heterogeneity of target endpoints, effect parameters and test design. The feeding inhibition of *daphnids* has significant potential as a sensitive sub-lethal endpoint in toxicity testing, however, in order to fully utilize it as a consistent tool in ecotoxicology, some level of standardization is required. This would allow comparison of test results generated in different laboratories as well as study of chemicals with different modes of action in terms of their influence on feeding activity. The sensitivity of feeding inhibition as a toxicity endpoint depends on the toxicity mechanism of the studied chemical. Based on the scarce data available to date, it appears that neurotoxic chemicals are relatively more potent inhibitors of *Daphnia* feeding compared to energy metabolism inhibitors and other chemicals.

CRediT authorship contribution statement

Villem Aruoja: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Juris Tunēns:** Writing – original draft, Formal analysis, Data curation. **Anne Kahru:** Writing – review & editing. **Irina Blinova:** Writing – review & editing, Writing – original draft. **Margit Heinlaan:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors, Villem Aruoja, Juris Tunēns, Anne Kahru, Irina Blinova and Margit Heinlaana declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35213.

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