Behavior as response parameter

A literature review
on the relevance for population sustainability

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1 Introduction

Since the early 1970s, contaminants have been shown to affect virtually every aspect of behavior of terrestrial and aquatic organisms (Little, 1990). In the same editorial (addressing papers from the Behavioral Toxicology Symposium in November 1987, Florida), Little stated that "the predictive potential of behavioral data will not be fully realized until we can relate behavioral changes to ecological effects". He suggested that a few monumental field studies might be sufficient to confirm what behavioral toxicologists continually assume to be intuitively obvious: that changes in behavior are reflected at the population and community levels.

Meanwhile, several steps have been taken. A few standardized protocols have become available providing guidance on behavioral toxicity tests with a limited number of organisms (fish, earthworms and springtails; ASTM E1711-12 & 1768-95; ISO 17512-1 & -2) and several technological developments stimulated automate data recording and computerized analyses (e.g. infrared-light; magnetic inductance, telemetry, computer-aided video tracking and impedance conversion technology). Furthermore, several behavioral tests have been developed for continuous monitoring of water quality including fish, bivalves, daphnids (Penders, 2011), gammarids and stoneflies (Gerhardt, 2007). However, linking behavior to biological effects on the organismal or population level is still a point of concern (Gerhardt, 2007; Melvin and Wilson, 2013).

1.1 Behavioral toxicity in risk assessments

Ecological risk assessments are conducted to protect ecosystems from anthropogenic activities, including the release of chemicals into the environment. The basic data for establishing acceptable environmental concentrations of chemicals are generated using standard laboratory toxicity tests in which the effects on mortality, growth and reproduction are assessed for a limited number of individual species (Brooks *et al.*, 2009). Although this approach might suffer from several constraints (such as mixture toxicity, interactions between species and more sensitive suborganismal parameters), it is still the preferred approach specified in several guidance documents (ECHA, 2012; EC, 2011). For example, the Technical Guidance for deriving environmental quality standards for the Water Framework Directive (EC, 2011) states that "*Studies used for EQS derivation should be those where the test endpoint can be related to ecologically significant hazards. For practical purposes, this means effects that can be linked to population sustainability and particularly: a) survivorship of adults, b) time taken to develop (particularly to reach reproductive age) and c) reproductive output*". In addition, this guidance states that "*the assessor may be faced with data from studies describing endpoints that do not include direct measurements of survival, development or reproduction but, rather, describe e.g. behavioral effects, anatomical differences between control and treatment groups, effects at the tissue or sub-cellular level, such as changes in enzyme induction or gene expression. Generally these are unsuitable as the basis for EQS derivation. However, some other endpoints are relevant. For example, anatomical changes to gonad development that would prevent successful reproduction, or changes in behavior if the effect described would impair competitive fitness may be relevant*". As this list is not exhaustive, both ECHA (2012) and EC (2011) provide as criterion that toxicity test results based on endpoints, whose relationship to effects at the population level is uncertain, are not included in the risk assessment and behavioral responses are mentioned as an example.

On the other hand, several studies state that behavioral responses are more sensitive endpoints than survival, with, for example, changes in locomotory behavior in fish occurring at 0.7 to 5% of the LC_{50}-value and at concentrations that subsequently inhibited growth after longer periods of exposure (Little and Finger, 1990). In addition, behavioral responses can have ecological effects at the population and community level (Weis and Candelmo, 2012) and can incorporate additional effects from species-interactions (e.g. predator–prey interactions; e.g. Brooks *et al.*, 2009; Floyd *et al.*, 2008). Finally, depending on the life history and developmental characteristics of different model species, assessing chronic endpoints can require a substantial amount of time and costs. With the wide range of environmental contaminants finding their way into aquatic environments there is a growing need for monitoring tools that are fast and sensitive to a wide range of compounds, but also indicative of potential effects on survival, growth and fitness (Melvin & Wilson, 2013). Behavioral analyses show promise for satisfying these requirements, and are often hailed for their rapidity (Bitton *et al.*, 1996; Diamond *et al.*, 1990; Gerhardt, 2007; Maradona *et al.*, 2012) and sensitivity (Amiard-Triquet, 2009; Little and Finger, 1990; Little *et al.*, 1993) compared to traditional toxicological methods assessing developmental and reproductive effects.

Goals of the present research

As illustrated above, behavioral toxicity offers several possibilities for a further improvement of environmental risk assessments but such use is hampered by the fact that a clear relationship with effects at the population level is not generally accepted. The present literature review, with an exploratory character, is therefore aimed at answering three different questions:

* Which behavioral parameters are frequently encountered in ecotoxicological studies?
* Are behavioral parameters comparable in their sensitivity to acute lethality, developmental and reproductive parameters?
* Which evidence exist providing a clear insight in the ecological relevance of behavioral parameters?

The results are reported and interpreted in the following chapters:
Chapter 2: A general overview of the available literature with special emphasis on behavioral parameters used, their role in standard test guidelines and their sensitivity compared to more traditional endpoints.
Chapter 3: A more detailed review on the ecological relevance of behavioral parameters.
Chapter 4: Concluding remarks.

2 Behavior as response parameter

An overview of the parameters and organisms in behavioral toxicity studies is provided in §2.1, using the EPA-ecotox database as source (http://cfpub.epa.gov/ecotox). Furthermore, standard test guidelines from OECD, ASTM or ISO were assessed to see in which behavioral toxicity was mentioned as test parameter, either as qualitative or quantitative parameter (§2.2). Finally, the literature was searched for studies in which the sensitivity of behavioral parameters was compared to acute lethality and especially chronic effects on growth or reproduction (§2.3).

2.1 General overview

In 1990, Little already concluded that behavioral toxicity was a growing discipline. Since then, a huge amount of papers have been published in which the toxic effects of >1000 different substances on several hundred different organisms are studied. As starting point for the present review, the EPA-Ecotox database (Aquire and Terretox) was searched for all toxicity data belonging to the effect parameter "behavior". This resulted in 18,246 data points between 1985-2014 originating from 2749 different references. The data are classified according to the organism group (Figure 2.1a) and the type of behavior (figure 2.1b).

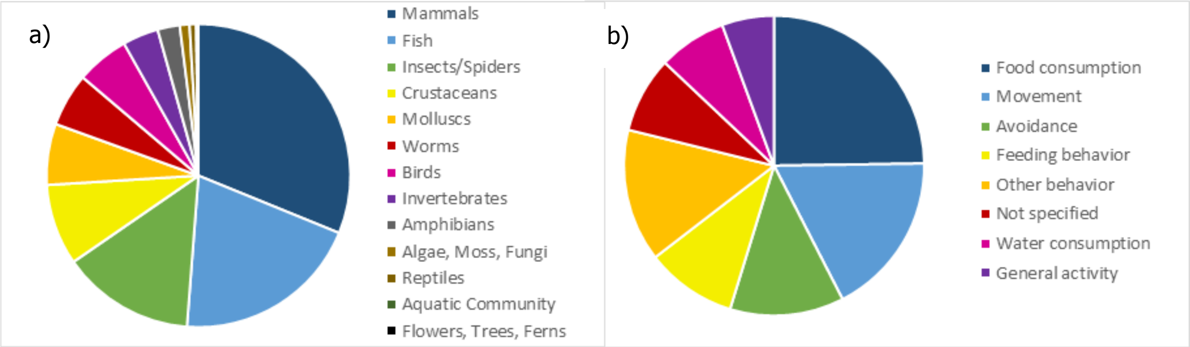

Figure 2.1. Representation of the 18,246 data points for "behavior" in the EPA-Ecotox database between 1985-2014 classified according to the organism group (figure a) and the parameter "effect measurement" to indicate the type of behavior in more detail.

Figure 2.1 illustrate that 50% of the data belong to mammalian and fish studies, with insects and crustacean as the third and fourth most frequently studied group of organisms. Furthermore, most of the behavioral activities relates to food consumption and feeding (effect parameters such as feeding efficiency, filtration rate, predator behavior) or to movement (e.g. swimming, avoidance, distance moved, burrowing). As to be expected, the behavioral parameters studied are not evenly distributed over the organisms (table 2.1). For small animals like insects, movement and avoidance is more easily being studied compared to feeding behavior, while the opposite holds for studies with mammals and birds.

Table 2.1. General description of the behavioral parameters studied among the different groups of organisms.

Organism	Aquatic			Terrestrial		
	Avoidance & movement	Food & water consumption	Other	Avoidance & movement	Food & water consumption	Other
Mammals				1103	3792	793
Birds				192	669	165
Reptiles	6		3	14	33	71
Amphibians	259	43	59	42	3	25
Fish	1233	1325	1099			
Insects/spiders	275	77	121	1373	416	323
Crustaceans	449	398	676	22	31	
Molluscs	431	329	382	23	23	
Worms	222	30	143	336	241	66
Invertebrates	350	195	85	27	40	
Algae, moss, fungi	125		44	4		17
Flowers, trees, ferns		1	9	8		
Aquatic community			25			

2.2 Behavior as parameter in standard toxicity tests

Toxicity data obtained according to standard test guidelines, such as OECD or ISO, are favored in environmental risk assessments. It was therefore questioned to what extent "behavior" is mentioned as test parameter in these guidelines. For this purpose the overview presented by Kramer *et al.* (2011) was extended to represent the type of behavior in more detail, to distinguish between guidelines in which behavior is the key-parameter and guidelines in which behavior is mentioned as additional parameter and to include other type of behaviors mentioned in the scientific literature (Table 2.2). This table does not intend to be complete but to provide an overview of guidelines often used in standard toxicity tests (excluding mammals and birds) as well as other examples from behavioral studies in literature.

From this overview it is noted that most standard test guidelines only mention that "abnormal behavior should be reported". They don't specify observation frequencies, nor mention any obligation to specify results per replicate or to quantify abnormal behavior in some way. For as far as abnormal behavior is noted in these standard toxicity tests, results will be mostly qualitative of nature and hardly useful in risk assessments. In addition to these standard guidelines, the literature search was extended to guidelines in which behavior is proposed as (one of the) key-parameters. For fish two guidelines exist varying from a broad spectrum guideline (ASTM E1711-12) to the more specified ASTM 1768-95, focusing on ventilatory behavior. In addition to these guidelines for fish, two guidelines were found for behavioral studies in invertebrates (ISO 17512-1 and 17512-2 studying avoidance in earthworms and springtails respectively) as well as the broad guideline ASTM E1604-12 providing a "standard guide for behavioral testing in aquatic toxicology".

In addition to these standardized guidelines, for each organism group several examples are incorporated in table 2.2 to illustrate the diversity of behavioral parameters used in literature. The extent to which these parameters are suited for standardization will vary. At the same time, the abundance of examples also illustrate that several frequently used parameters offer sufficient possibilities for further standardization such as valve movement and filtration for bivalves, phototactic and feeding in daphnids, feeding inhibition in amphipods or accuracy of learned tasks for honeybees.

Table 2.2. Overview of terrestrial and aquatic tests guidelines in which behavior is mentioned as test parameter.

Organism	Parameter	Reference
Amphibians		
	Guidelines in which behavior is used as additional aspect	
	”Cases of abnormal behavior, grossly visible malformations and lesions should be recorded. Abnormal behavior would include, floating on the surface, lying on the bottom of the tank, inverted or irregular swimming, lack of surfacing activity, and being nonresponsive to stimulus.”	ASTM E2591-07, OECD 231, OPPTS 850.1800
	Guidelines in which behavior is used as key parameter	
	-	
	Other examples from literature	
	Avoidance	Rohr & Crumrine, 2005; Berrill *et al.*, 1998; Storrs Mendez *et al.*, 2009
	Feeding behavior	Zaya *et al.*, 2011; Gurushankara *et al.*, 2007; Park *et al.*, 2001
	Movement	Denoel *et al.*, 2010; Helbing *et al.*, 2006; Mitchkash *et al.*, 2014
	Predator-prey	Bridges 1999; Squires *et al.*, 2008; Relyea & Edwards, 2010
	Response to a stimulus	De Jong Westman *et al.*, 2010; Helbing *et al.*, 2006; Wacksman *et al.*, 2006
	Swimming	Carr *et al.*, 2003; De Arcaute *et al.*, 2012; Punzo & Parker, 2005
Fish		
	Guidelines in which behavior is used as additional aspect	
	”abnormalities, e.g. hyperventilation, uncoordinated swimming, and atypical quiescence should be recorded at adequate intervals depending on the duration of the test”	ISO 10229, OECD 203, 204, 210, 212, 215, 229, 230, OPPTS 850.1400
	Guidelines in which behavior is used as key parameter	
	Behavior	ASTM E1711-12; ASTM E1604-12
	Ventilatory behavior	ASTM E1768-95
	Other examples from literature	
	Accuracy of learned task	Carvan *et al.*, 2004; Levin *et al.*, 2003; Timme-Laragy *et al.*, 2006
	Aggression	Colman *et al.*, 2009; McDonald *et al.*, 2011; Saaristo *et al.*, 2010
	Avoidance	Richendrfer *et al.*, 2012; Labenia *et al.*, 2007; Tierney *et al.*, 2007
	Feeding behavior	Stara *et al.*, 2012; Welker *et al.*, 2012; Floyd *et al.* 2008
	Migration	Moore *et al.*, 2008; Scholz *et al.*, 2000; Teather *et al.*, 2005
	Motility	Barimo & Walsh, 2005; Bowman *et al.*, 2012; Carlsson & Norrgren, 2004
	Movement	Kienle *et al.*, 2009; Senger *et al.*, 2011; Carlsson *et al.*, 2013
	Phototactic response	Lorenz *et al.*, 1996; Steinberg *et al.*, 1995
	Predator-prey	McGee *et al.*, 2009; Palm & Powell, 2010; Gutierrez *et al.*, 2013
	Response to a stimulus	Huang *et al.*, 2010; Schultz *et al.*, 2012, Van der Schalie *et al.*, 2004
	Swimming	Boyle *et al.*, 2013; Campbell *et al.*, 2002; Tilton *et al.*, 2011

Table 2.2. Continued

Organism	Parameter	Reference
Molluscs		
	Guidelines in which behavior is used as additional aspect	
	-	
	Guidelines in which behavior is used as key parameter	
	-	
	Other examples from literature	
	Ability to detach from substrate	Angarano *et al.*, 2007; Ericson *et al.*, 2010; Sanchez-Lazo & Martinez-Pita, 2012
	Avoidance	Kalil-Gaspar, 2007; Oplinger & Wagner, 2009; Vyskushenko, 2006
	Burrowing	Flynn *et al.*, 2013; Hutchins *et al.*, 2008; Stockman, 2008
	Feeding	Brix *et al.*, 2012; Cheung *et al.*, 2002; Coeurdassier *et al.*, 2001
	Filtration rate	Mouabad *et al.*, 2001; Neuberger-Cywiak *et al.*, 2007, Yu *et al.*, 2010
	Foot retraction	Bringolf *et al.*, 2007; Pandolfo *et al.*, 2012; Rajagopal *et al.*, 1997
	Movement	Bernot *et al.*, 2005; Gerard & Poullain, 2005; MacFarlane *et al.*, 2004
	Valve closure	Curtis *et al.*, 2000; Faria *et al.* 2010; Liao *et al.*, 2009
Crustaceans		
	Guidelines in which behavior is used as additional aspect	
	"Any abnormal behavior or appearance such as trapping at surface of water should be reported."	ISO 14669, OECD 202, 211
	Guidelines in which behavior is used as key parameter	
	-	
	Other examples from literature	
	Avoidance	Lopes *et al.*, 2004; Loureiro *et al.*, 2009; Rosa *et al.*, 2012
	Burrowing	Hecht & Boese, 2002; Pynnonen, 1996; Weis & Perlmutter, 1987
	Feeding	Bitton *et al.*, 1996; Reynaldi *et al.*, 2006; Drobne *et al.*, 2008
	Movement	Guler & Ford, 2010; Norum *et al.*, 2010, Vellinger *et al.*, 2013
	Phototactic response	Kolkmeier & Brooks, 2013; Martins *et al.*, 2007; Yuan *et al.*, 2003
	Predator-prey	Bundschuh *et al.*, 2012; Gutierrez *et al.*, 2013; Santos *et al.*, 2000
	Swimming	Alonso *et al.*, 2009; Hellou *et al.*, 2009; Rao *et al.*, 2007
Insects and arachnids		
	Guidelines in which behavior is used as additional aspect	
	"The test vessels should be observed at least three times per week to make visual assessment of any abnormal behavior (for example leaving sediment, unusual swimming), compared with the control"	OECD 218, 219, 233
	Guidelines in which behavior is used as key parameter	
	Avoidance	ISO 17512-2

Table 2.2. Continued

Organism	Parameter	Reference
Insects and arachnids-continued		
	Other examples from literature	
	Accuracy of learned task	Aliouane *et al.*, 2009; Decourtye *et al.*, 2005; Le Bourg, 2007
	Aggression	Pankiw, 2009; Stevenson *et al.*, 2005; Schneider *et al.*, 2003
	Avoidance	Dornfeld *et al.*, 2009; Owojori *et al.*, 2011; Zhou *et al.*, 2010
	Feeding	Alexander *et al.*, 2007; Brewer & Atchison, 1999; Koul *et al.*, 2004
	Movement	Kim *et al.*, 2006; Prasifka *et al.*, 2008; Van Gossum *et al.*, 2009
	Predator-prey	Cloyd *et al.*, 2009; De Castro *et al.*, 2013; St Clair & Fuller, 2014
	Response to a stimulus	Bayram *et al.*, 2010; El Hassani *et al.*, 2005; Reynaldi *et al.*, 2011
	Swimming	Mehler *et al.*, 2008; Perez *et al.*, 2013; Schuler *et al.*, 2005
	Tube Building	Balch *et al.*, 2000; MacDonald *et al.*, 2004; Schulz *et al.*, 2001
Annelids		
	Guidelines in which behavior is used as additional aspect	
	"Any unusual behavior (e.g. inability to dig into the soil/sediment; lying motionless; avoidance, which may be caused by the test chemical, fecal pellets visible on the sediment surface) are recorded"	OECD 207, 222, 225; ISO 11268-2
	Guidelines in which behavior is used as key parameter	
	Avoidance	ISO 17512-1
	Other examples from literature	
	Avoidance	Amorim *et al.*, 2008; Loureiro *et al.*, 2006; Santos *et al.*, 2012
	Burrowing	Buffet *et al.*, 2011; Capowiez *et al.*, 2006; Leland *et al.*, 2001
	Feeding	Capowiez *et al.*, 2010; Lahr *et al.*, 2008; Moreira *et al.*, 2005
	Movement	Knakievicz & Ferreira, 2008; O'Gara *et al.*, 2006; Gerhardt, 2009
Nematoda		
	Guidelines in which behavior is used as additional aspect	
	Not mentioned	ISO 10872
	Guidelines in which behavior is used as key parameter	
	-	
	Other examples from literature	
	Feeding	Anderson *et al.*, 2001; Boyd *et al.*, 2007; Gomez-Eyles, 2009
	Movement	Anderson *et al.*, 2004; Dhawan *et al.*, 2000; Rajini *et al.*, 2008
	Predator-prey	Gutierrez *et al.*, 2008; Head et; 2000; Siroha & Siyanand, 1994

2.3 Is behavior a more sensitive parameter?

It is often mentioned that behavior is a particularly sensitive measure of an organism's response to stress, including environmental contaminants (Weis and Candelmo, 2012) and noticeable changes in behavior can be found at concentrations of chemicals that are orders of magnitude below those that can cause mortality (Gerhardt, 2007; Robinson, 2009). These statements are supported by numerous studies covering for example fish (Little and Finger, 1990), marine polychaetes and bivalves (Bonnard *et al.*, 2009), daphnids (Martins *et al.*, 2007; Reynaldi *et al.*, 2006), snails (Bernot *et al.*, 2005), chironomids, (Janssens-de Bisthoven *et al.*, 2004), amphipods (Felten *et al.*, 2008), freshwater polyps (Quinn *et al.*, 2008) and honeybees (Aliouane *et al.*, 2009) all demonstrating that behavioral impairments occur at sublethal concentrations. However, exceptions also exist (Allen *et al.*, 1995; Araujo *et al.*, 2014; Bitton *et al.*, 1996; Gray, 1990).

While such comparisons with acute lethal concentrations are frequently found, comparisons with chronic sub-lethal effects on growth or reproduction are more scarce. Most of the studies indicate that concentrations at which acute effects on behavior occur, are more or less comparable to chronic effect levels for growth or reproduction. This applies for example to larvae of the fathead minnow and esfenvalerate exposure (Floyd *et al.*, 2008), a simultaneous exposure of TiO_2-nanoparticles and UV light on *Gammarus fossarum* (Kalcikova *et al.*, 2014), esfenvalerate exposure of the amphipod *Gammarus pulex* (Cold and Forbes, 2004), or several toxicants for *Daphnia magna* (Allen *et al.*, 1995; Reynaldi *et al.*, 2006) or the terrestrial mite *Oppia nitens* (Owojori *et al.*, 2011). Again exceptions do exist (Flickinger *et al.*, 1982; Kolkmeier and Brooks, 2013) but even in these cases good correlations can still be found (Domene *et al.* [2007] and Mommaerts *et al.* [2010] studying collembolan resp. bumblebee feeding and reproduction).

It is interesting to see that differences in sensitivity might be caused by the physical-chemical characteristics of the toxicant: Using different compounds the experimental results of Allen *et al.* (1995) (studying daphnid feeding behavior) supported the hypothesis that, while all compounds were capable of causing feeding inhibition, electropositive species such as cadmium induced effects close to the chronic no-effect concentration, whereas electronegative species such as vanadium induced effects only at or close to lethal levels.

These references relate to studies in which effects on behavior, mortality, growth or reproduction are simultaneously studied. In other studies a comparison is made to ecotoxicological effects as observed in other studies. For example, Pedersen *et al.* (2013) studied the effects of a 1h pulse of permethrin on the behavior and reproduction of adult *H. Azteca* and compared the data with unpublished experiments, in which juveniles were exposed to comparable concentrations. They concluded that a behavioral response occurring within minutes after exposure (the separation of precopulatory pairs of *Hyalella*) and the reduced reproductive output in the following 56 days after this pulsed exposure were more sensitive parameters compared to the standard toxicity test in which juveniles were exposed.

In addition to these individual studies Melvin and Wilson (2013) performed a meta-analysis in which the relative sensitivities and average durations of behavioral studies were compared to those assessing acute lethality, development and reproduction in aquatic toxicity testing. They addressed four different research questions; 1) Are the average timeframes of behavioral experiments comparable to those of acute lethality, developmental and reproductive experiments? 2) Are behavioral experiments comparable in their sensitivity to acute lethality, developmental and reproductive experiments? 3) Are response magnitudes comparable amongst studies assessing behavioral, developmental, and reproductive endpoints? and 4) Does the statistical power achieved with behavioral studies compare to that of studies assessing developmental and reproductive endpoints? They performed an extensive literature search and excluded all contaminant – class of organism combinations where information did not exist for all four types of study (i.e. behavioral, acute LC50, development and reproduction). The only studies that met this criterion were those with fish and crustaceans as the model organisms for which 106 data points from 60 studies describing 11 different contaminants for crustaceans, and 220 data points from 133 studies describing 14 contaminants for fish were recovered. Several behavioral parameters were included such as swim speed, distance moved, activity levels, spatial distribution patterns, feeding rates and courting events. As expected the timeframe (days) required for behavioral studies (1.58 ± 5.25) and those assessing acute lethality (3.46 ± 1.02) was significantly lower than studies assessing developmental (43.54 ± 68.03) or reproductive endpoints (44.34 ± 69.45) and acute lethality estimates were the least sensitive parameter. Melvin and Wilson (2013) also concluded that overall behavioral studies were more sensitive than those assessing developmental and reproductive endpoints (figure 2.2). For studies with crustaceans as the model organism only, there was however no statistical difference in the sensitivity of behavioral, developmental or reproductive studies. Conversely, behavioral responses had greater sensitivity than those investigating developmental or reproductive endpoints in studies with fish as the model organism. Exceptions did however also exist. For example, behavioral studies with crustaceans were less sensitive than developmental or reproductive studies for lindane and zinc and behavioral studies with fish were less sensitive than developmental or reproductive studies for cyanide and mercury. These comparisons of study duration, sensitivity, effect size and statistical power indicate, according to Melvin and Wilson (2003), that behavioral responses should be more widely utilized from both a statistical and financial point of view. However, Melvin and Wilson also stressed that for behavioral studies to offer the most meaningful assessment of toxicological risk, research must be focused towards understanding how changes to discrete animal behaviors relate to broader ecological concerns such as survival, health and fitness.

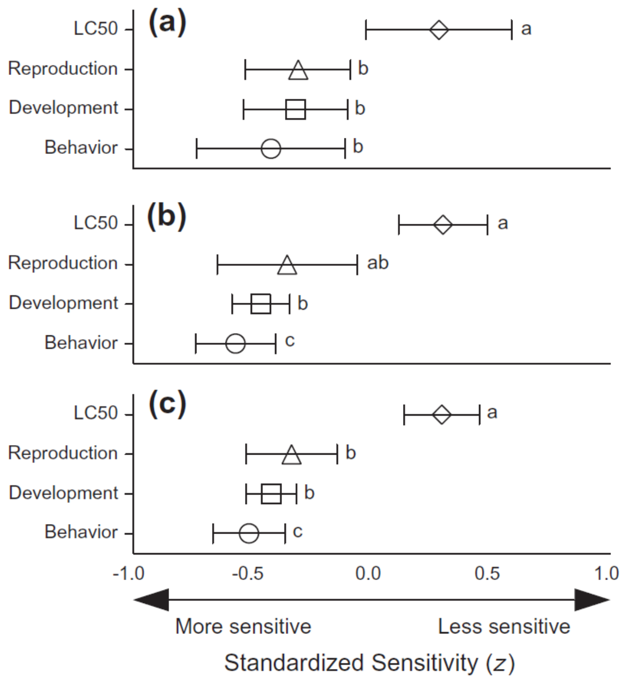

Figure 2.2. Mean sensitivity scores (z-scores) of studies with (a) crustaceans and (b) fish as the model organisms, and (c) overall z-scores, for studies assessing acute lethality, behavioral, developmental and reproductive endpoints. z-scores were calculated independently for each contaminant where information existed for all study types. Bars represent 95% confidence interval. Different letters indicate significant differences ($a < 0.05$) based on 10000 random permutations of the data. Figure copied from Melvin and Wilson (2013).

When behavioral parameters are indeed slightly more sensitive compared to effects on growth and reproduction, the inclusion of behavioral parameters in risk assessments might result in somewhat lower EQS values. On the other hand, inclusion of behavioral parameters might extend the number of tested organisms and in doing so lower the safety factors, which in turn will result in the opposite effect and increasing EQS values.

Conclusions

* Over the last three decades (1985-2014) several thousand papers are published describing toxic effects on behavioral parameters for several hundred different organisms. Around 50% of the data belong to mammalian and fish studies, with insects and crustacean as the third and fourth most frequently studied group of organisms. Most of the behavioral activities relates to food consumption and feeding (effect parameters such as feeding efficiency, filtration rate, predator behavior) or movement (e.g. swimming, avoidance, distance moved, burrowing).
* Most standard test guidelines mention the obligation to report "abnormal behavior", but the results will be mostly qualitative of nature and hardly useful in risk assessments. Only a limited number of guidelines exist in which behavior is proposed as (one of the) key-parameters. Excluding mammalian and bird studies, only two

ASTM-guidelines for fish studies and two ISO-guidelines for earthworm and springtail avoidance were found. At the same time, several frequently used parameters offer sufficient possibilities for further standardization, such as valve movement and filtration for bivalves, feeding in daphnids or amphipods or accuracy of learned tasks for honeybees.

* Behavioral responses are in general (much) more sensitive than acute lethality. It is less certain whether this also holds for chronic effects on growth or reproduction. A meta-analysis for fish and crustacean demonstrated that overall behavioral studies were somewhat more sensitive than those assessing developmental and reproductive endpoints. However, this difference was not found when the crustacean studies were separately tested. As working hypothesis it seems therefore reasonable to assume equal sensitivity between behavioral, growth and reproductive parameters.
* If behavioral parameters are indeed slightly more sensitive compared to effects on growth and reproduction, the inclusion of behavioral parameters in risk assessments might result in somewhat lower EQS values. On the other hand, inclusion of behavioral parameters might extend the number of tested organisms and in doing so lower the safety factors, which in turn will result in the opposite effect with increasing EQS values.

3 The ecological relevance of behavioral responses

There are several ways to validate the hypothesis that behavioral responses can have a clear relation with population sustainability as the TGD-EQS (EC, 2011) mentions that such a relation might either be a definite correlation or a causal relationship.

1. *Experiments might combine behavioral responses with simultaneously studied effects on growth, reproduction or mortality*
   Individual experiments can elucidate the ecological relevance of behavioral responses and toxicants. Several examples are mentioned in §2.3. Extrapolation of these individual studies to the ecological relevance of behavioral responses in general will however be difficult. Furthermore, this approach is less useful for the present review focusing on risk assessments, as only two possible outcomes exist. When behavioral responses in individual studies are as sensitive or less sensitive compared to mortality, growth or reproduction, the risk assessment will not differ from a situation in which the behavior was not included. When behavioral responses are more sensitive compared to growth or reproduction, the individual experiment itself is insufficient to demonstrate a clear relation with population sustainability, although such a relation can off course still exist. In such a case other evidence will be needed before behavioral parameters of such an individual study will be included in the setting of environmental quality criteria. For example, multi-generation experiments indicating that effect levels on growth and reproduction decrease in consecutive generations, may confirm that sensitivity towards a toxicant is higher than initially considered on the basis of a standard test.

2. *The relationship might be demonstrated by combining several papers studying either behavioral or mortality, growth and reproduction responses within the same toxicant-organism combination*
   This argument holds when a significant relation can be established between behavioral parameters and mortality or chronic effect levels for growth or reproduction, covering large amounts of toxicants and (preferably) different groups of organisms. Numerous individual articles were found discussing this subject (see §2.3), but especially the meta-analysis performed by Melvin and Wilson (2013; also discussed in §2.3) provides such an example. Based on their results it can be concluded that a correlation exists between behavioral responses and chronic effect levels for growth or reproduction within fishes and crustacean. The strength of this statement will increase when comparable analyses come available for other groups of organisms or with an extended number of toxicants. In the meantime, as a definite correlation is sufficient according to the TGD-EQS (EC, 2011), it might be stated that an experiment studying only behavioral responses for fish or crustacean could be included in a risk assessment without further supporting evidence for population level effects.
   In such case, the next question will automatically be the selection of appropriate Assessments Factors. Melvin and Wilson (2013) demonstrated that overall behavioral studies were more sensitive than those assessing developmental and reproductive endpoints. At the same time these differences were not statistically significant when crustaceans were studied only, while the 95% conf. limits for fishes strongly overlapped between these parameters. Compared to the normally observed large differences in toxicity between species, exposure durations and age of the test organisms, these differences might be considered small. In such a case it can be argued that the standard AF scheme might still be applicable for which the behavioral toxicity data can be added as an additional test species.

3. *Ecotoxicological experiments might combine behavioral responses with simultaneously studied effects on parameters with a distinct effect on population sustainability (excluding mortality, growth or reproduction)*
   Multispecies experiments in which predation of exposed prey is studied would normally not be used in risk assessments. The same applies for algal growth rate when the filter feeder is exposed to toxicants. At the same time, these experiments might elucidate how behavioral impairments might affect both populations and communities. The results of the present inventory are discussed in §3.1.

4. *Large-scale case studies in which several years of research is combined into a comprehensive understanding of behavioral responses and their consequences for populations and communities*
   By nature, the number of these studies will be limited. At the same time, case-studies combining several laboratory and field studies, might just be the kind of "monumental field studies" requested by Little (1990) "to confirm what behavioral toxicologists continually assume to be intuitively obvious: that changes in behavior are reflected at the population and community levels". The results of the present inventory are discussed in §3.2. This also includes some reviews.

5. *Consequences of behavioral impairments for individual fitness or population sustainability can be discerned by combining ecotoxicological studies with ecological research on the role of this behavior in natural population dynamics (without the stress of toxicants)*
   From way back ecologist have been studying the behavior of animals in relation to their individual fitness or population sustainability. This might form sufficient evidence to establish the relation between toxicant induced behavioral responses and effects on population or community level. The results of the present inventory are discussed in §3.3.

In §3.1, 3.2 and 3.3 individual studies are summarized indicating the organism, the type of behavior studied as well as its ecological consequence. Based on the exploratory character of the present literature review, this overview is far from being complete. It intends to provide insight in the ways to validate the hypothesis that behavioral responses can have a clear relation with population sustainability. Still, the present overview supports some more general conclusions and recommendations. These are presented in chapter 4.

3.1 Multi-species tests in the laboratory

Ecologists have published dozens of articles on a high diversity of species demonstrating the relationships between the behavior of prey species and the risk of predation. A reduced activity often provides fewer visual cues for active hunters to locate their prey, important for eliciting a predator attack (Greene, 1986) and reduce encounter rates with ambush predators. For example, the attack rate of the ambush predator *Chaoborus americanus* decreased as the swimming speed, and thus encounter rates of its *Daphnia* prey decreased (Spitze, 1985), while bluegill sunfish preferentially selected daphnids showing "spinning" behavior which can be caused by carbaryl exposure (Dodson *et al.*, 1995). Prey choice of a predatory stonefly *Hesperoperla pacifica* was affected by the activity of two prey species (Molles and Pietruszka, 1983). A decrease in tadpole activity can result in lower predation rates through reduced detection by visually oriented predators (Lawler, 1989) and in more general terms evasive behavior is a primary survival strategy for many organisms (Vamosi and Schluter, 2002). It can therefore be expected that contaminants affecting the behavior of prey species, will also cause indirect effects on predator-prey relationships, which in turn can have consequences on the mortality rates of the prey species. The literature was therefore searched for examples in which contaminants caused such indirect effects on predator-prey relationships. Several examples are discussed below, covering fish larvae, aquatic insects, daphnids, rotifers and isopods.

Predator-prey interactions (review)
Preston (2002) reviewed the scientific literature on the indirect effects of toxicants and concluded that as indirect effects are a major consideration in ecology, they should also be a consideration in ecotoxicology and ecological risk assessment. He considered predator-prey relations in more detail and summarized several articles on this subject, such as (references can be found in Preston, 2002)
i) Clements *et al.* (1989), who observed that caddisfly (*Hydropsyche morosa*) predation by stoneflies (*Paragnetina media*) in experimental microcosms increased significantly after sublethal exposure to copper, presumably due to toxicant-induced changes in predator avoidance behavior of *H. morosa*.

ii) Tagatz (1976), who observed that mirex increased swimming activity of grass shrimp (*P. vulgaris*) and pinfish (*Lagodon rhomboides*), causing increased visibility to predators.
iii) Gomez *et al*. (1997), who examined the effects of sublethal PCP exposure on the predator–prey interaction between *B. calyciflorus* and the predatory rotifer *Asplanchna girodi* and found that PCP reduced *A. girodi* capture and ingestion efficiency. In addition, Preston *et al*. (1999) studied the effects of PCP on the vulnerability of several rotifer species to predation by *A. girodi* and demonstrated that due to differential responses of prey species to PCP, changes in prey vulnerability after exposure varied among prey species.

Qualitatively, these examples (and several others) are sufficient to illustrate a relation between behavioral responses in activity or movement and individual fitness or population level effects. According to the EC (2011) this might be sufficient to include effects on these behavioral parameters in future risk assessments. At the same time, Preston (2002) also stated that so far little attempt has been made to quantitatively assess the impacts at higher levels of biological organization. He mentioned an earlier publication of himself and co-workers in which mathematical models were used to estimate the effects of toxicant-induced changes in the swimming behavior of *B. calyciflorus* on the risk of ingestion by *A. girodi* over the course of its estimated lifespan. Such an approach allows one to use laboratory data to make ecologically relevant predictions regarding the fate of natural populations. More recently, Preston and Snell (2000, reviewed in Preston, 2002) used a similar approach to compare the relative effects of toxicant-induced changes in predation risk versus toxicant-induced changes in reproduction for several species of rotifer exposed to sublethal PCP concentrations. Results indicated that toxicant effects on predator–prey interactions were negligible when compared to toxicant effects on reproduction, suggesting single-species toxicity data at the population level may be useful in examining toxicant effects on species interactions. It is yet hard to tell whether this is just an example or an illustration of a wider applicable difference in sensitivity at the population level. For risk assessments this is however less important; when reproduction is the more sensitive parameter, making use of behavioral studies in environmental risk assessments will not overestimate toxicity. When the opposite holds and predator-prey relations are more sensitive, environmental risk assessments can only benefit by making use of behavioral studies on movement and predator avoidance.
More recently several other examples of predator-prey relations have been published:

Fish larvae; movement; predation
An abnormal swimming behavior was observed by larvae of the fathead minnow *Pimephales promelas* after a 4hrs exposure to the pyrethroid insecticide esfenvalerate (Floyd *et al*., 2008). Abnormal swimming behavior included twitched and erratical swimming as well as reduced swimming activity. The ecological consequences of these behavioral impairments were additionally studied in a 45 minutes predation experiment, in which the number of minnow larvae predated by threespine stickleback *Gasterosteus aculeatus* was assessed. This experiment demonstrated that minnow larvae became more vulnerable to predation as pesticide concentration increased and fathead minnows exposed to the high esfenvalerate concentration exhibited higher predation risk than those exposed to the low concentration or the controls (Floyd *et al*., 2008). Comparable experiments were performed by Webber and Haines (2003) studying the effects of mercury on golden shiner exposed to a model avian predator indicating that dietary mercury exposure would increase vulnerability of the fish to predation.

Aquatic insects; movement; predation
Brooks *et al*. (2009) investigated the interaction between behavioral changes in prey and its predation by predators with different hunting strategies. Ambush (*Ischnura elegans*; Odonata) and active (*Notonecta glauca*; Heteroptera) predator species were used in conjunction with three prey species (the isopod *Asellus aquaticus*, the mayfly *Cloeon dipterum* and the midge larvae *Chironomus riparius*). Sublethal exposure of prey to cadmium did not affect the prey choice of active predators, possibly because of prey behavioral changes being insufficient to influence their relative availabilities. However, cadmium exposure of prey did alter their susceptibility to ambush predators. There was a reduced proportion of *C. dipterum* and an increased proportion of *A. aquaticus* in the diet of ambush predators, possibly because of reduced activity in *C. dipterum* affecting their relative encounter rates with predators. This shift in prey choice of the ambush predator species highlights one of the limitations of current ecological risk assessment practice: using a single-species approach would result in the conclusion that *C. dipterum* was not significantly affected by cadmium exposure, as activity was not significantly reduced. However, this subtle change in behavior was detected by predators and only in the more complex two-prey species system. The resultant shift in predation caused a negative impact on the otherwise unaffected *A. aquaticus* prey (Brooks *et al*., 2009).

Van *et al*. (2014) studied the predator and anti-predator behaviors of the damselfly *I. elegans*, when exposed to chlorpyrifos for 6 days. These authors demonstrated that both predator (e.g. feeding strikes on *Artemia*) and anti-predator (e.g. escape swimming speed) behaviors were reduced by chlorpyrifos exposure. This will likely result in reduced fitness of damselfly larvae as it decreased food intake and reduced escape burst speed, hence increased the probability of being killed by predation.

Aquatic insects; movement & feeding; predation
Riddell *et al*. (2005) studied foraging and predator-avoidance behaviors of mayfly (*Baetis tricaudatus*) nymphs under different combinations of cadmium concentration and predation risk. Experimental food webs were constructed using the diatom *Nitzschia* sp. as the primary producer, nymphs of the mayfly *Baetis tricaudatus* and the stonefly *Kogotus nonus* as invertebrate grazers and predators, respectively, and two fish species (*Salvelinus fontinalis* and *Rhinichthys cataractae*) as vertebrate predators. Both predators and cadmium produced significant effects on the behavior of mayflies and stoneflies, although these responses were complex to understand due to the number of different species-specific interactions. The feeding rate of mayflies, for example, increased by cadmium exposure in the absence of predators (maybe caused by an increased energy expenditure), while a decrease was observed in the presence of cadmium and stoneflies and almost no change in the presence of cadmium and the dace *R. cataractae*. This example illustrates that effect parameters for cadmium exposure, such as NOEC and EC_{50}-values, would differ depending on the predator presence. However, due to the number of species-specific interactions, the relation between behavioral responses and population sustainability was not unidirectional. An exception is formed by the significant decrease in capture efficiency of prey items when brook trout *S. fontinalis* was exposed to 0.5 and 5.0 mg/l Cd, which automatically would cause an increased population sustainability for their prey items.

Aquatic insects; movement and case-building; predation
Johnson *et al*. (2008) exposed caddisfly larvae (*Brachycentrus americanus*) to different sublethal concentrations of the insecticide esfenvalerate and determined the case-abandonment response, the ability to construct new cases and the risk of predation by stonefly nymphs. A 48-h exposure resulted in over 60% of larvae abandoning cases (none in control), a significantly reduced ability to construct new cases and an increased risk of predation. This increased predation risk was not only observed for caddisfly larvae, which had left their cases but also (somewhat weaker) for caddisfly larvae in rebuilt, weaker cases.

Crustacean; feeding; algal growth
Available ecological research extensively describe the effect of grazing rates by zooplankton on the population dynamics of phytoplankton as well as the additional influence of zooplankton eating fish (e.g. Jeppesen *et al*., 1998; Mills and Schiavone, 1982; Persson *et al*., 1992 cited in Bengtsson *et al*., 2004). Based on these insights, Bengtsson *et al*. (2004) studied the density-dependent grazing rate of *Daphnia pulex* pre-exposed to DDE (insecticide metabolite) and glyphosate (herbicide). As expected the growth rate of the algae *Scenedesmus* spp. was inversely related to the density of the grazer. In addition, a reduction of 30-40% in the grazing rate of *D. pulex* by pre-exposure to DDE resulted in a 60-70% increase in the growth rate of *Scenedesmus*. Sublethal effects on feeding behavior in daphnids can therefore not only affect the growth rate of the daphnids but also the growth of their food source. This kind of indirect effects between zooplankton grazing and phytoplankton growth are often observed in mesocosms studies with pesticides (Lucassen and Leeuwangh, 1993; Borgmann *et al*., 1989).

Isopods; movement; predation
Both Ham *et al*. (1995) and Bundschuh *et al*. (2012) studied the predator-prey relations between the isopod *Asellus aquaticus* and its predator the freshwater triclad turbellarian *Dendrocoelum lacteum*. The results are variable. Ham *et al*. (1995) found a decrease in predation risk by exposure to cadmium as did Bundschuh *et al*. (2012) during exposure to the fungicide tebuconazole. In contrast, exposure to the pyrethroid insecticide lambda-cyhalothrin increased predation success by 40% (although this increase was not statistically different). Bundschuh *et al*. (2012) discussed that both the decrease and increase in predation seemed to be primarily driven by an altered activity of the prey *A. aquaticus* as any shift in the prey’s activity influenced its probability to stick to mucus, a viscous substance released by *D. lacteum*, or to encounter the predator directly.

As illustrated above, numerous studies demonstrated adverse effects of toxicants on predator-prey relations in the laboratory. It is often hypothesized that these disrupted interactions will affect population sustainability of prey (reduced mortality risk) and predator (reduced food intake). It is however hard to verify this hypothesis under field conditions (or mesocosms as an intermediate). The effects of predation on prey population dynamics can be complex and unpredictable, largely because population control may also occur through bottom–up (e.g. food supply) and other top–down (e.g. social interactions and disturbance) ecological processes (Scott and Sloman, 2004). Predators often affect prey in a density-dependent fashion, so abundances of predator and prey populations can be tightly linked and predators may impact prey species to different extents, depending largely on the ability of prey to avoid predation.

Regarding the requirements for behavioral parameters in EQS-setting as specified by the TGD-EQS (EC, 2011), it can therefore be argued that these kind of laboratory predator-prey studies do not unequivocally demonstrate a relation between predator-prey interactions and population sustainability. At the same time, the same arguments holds for a reduced survival, growth or reproduction: quantitative, the effect under field conditions will be different and might even not occur at all. Within the present study other lines of evidence were therefore searched to add arguments to the discussion. First of all, field studies were searched to answer the question whether a disrupted predator-prey relation in the laboratory can be correlated to comparable processes in the field. The review by Weis and Candelmo (2012; see §3.2) provides such an illustration for fish. More of these studies will be valuable, although it will be hard to perform comparable research for smaller, invertebrate species. Another line of reasoning was therefore to look for mathematical models in which the effect of an increased predation cq. mortality on population dynamics can be simulated (§3.3).

3.2 Large-scale case studies & reviews

It is often mentioned that the ecological relevance of behavioral responses might be influenced by the length of the exposure (often short-term studies) as recovery might occur. Several authors have for example reported that food consumption by fish was initially reduced by exposure to different metals but partial or full recovery to control-level feeding occurred within 3 weeks (reviewed by Amiard-Triquet, 2009). Studies in which laboratory experiments are verified under field conditions can therefore add to our understanding and substantiate the ecological relevance. Several examples were found in which a behavioral impairment was assessed under both laboratory and field/mesocosm conditions (e.g. McWilliam and Baird, 2002 a,b; Norum *et al.*, 2010; Smith and Bailey, 1990). However, the examples mentioned below only focus on studies with a more distinct relation with population sustainability.

Fish; movement; predation

Extensive research on behavioral responses, predator/prey relationships and growth of two fish species has been carried out by Weis and co-workers as reviewed by Weis *et al.* (2001) with a follow up by Weis and Candelmo (2012). They combined laboratory studies on feeding behavior and toxic effects with field observations on the mummichog *Fundulus heteroclitus* and his predator the bluefish *Pomatomus saltatrix* living in contaminated estuaries to examine population and community level consequences. Contaminants in these estuaries include PCBs, PAHs, dioxins, pesticides and several metals.

Initial studies demonstrated that mummichogs from contaminated sites were less active and less able to capture prey (grass shrimp) than those from reference sites. Furthermore, the exposed mummichog population had a reduced life span and growth, for which the reduced feeding could be partly responsible. When fish from the reference site were kept in aquaria and exposed to sediments and food from the contaminated site, their prey capture ability decreased to be equal to that of the exposed population and the level of Hg in their brains increased to that of the exposed population. Stomach contents of field-caught fish from the contaminated estuary contained more sediment and detritus and less prey than fish from the reference site. Furthermore, exposed fish were less active and more vulnerable to predation by blue crabs than fish from the reference population. Their poor diet and poor predator avoidance could help explain earlier observations that these fish did not grow as well or live as long as fish from reference sites. In a large field study covering many different sites the proportion of grass shrimps in the stomach correlated well with the capture rate measured in the laboratory. In addition is was verified that the predator avoidance ability of the grass shrimp was not impaired as grass shrimp from the contaminated site were just as frequently captured as shrimp from the reference site. These behavioral changes in mummichogs were correlated with abnormal neurotransmitter levels (reduced serotonin), expanded thyroid follicles and reduced levels of T3, the active form of the thyroid hormone in fish which affect activity levels.

It is interesting to see that these behavioral changes in mummichogs also seemed to affect the population dynamics of grass shrimps. Bass *et al.* (2001) studied the *Palaemonetes pugio* populations in both the contaminated and reference estuaries and found that the grass shrimp were both larger in size and more numerous at the polluted site. Furthermore, laboratory studies in which juvenile shrimp from both populations were maintained in aquaria with sediments and water from both sites showed that exposed grass shrimp did not grow faster than unexposed shrimp. The larger size and greater population density of the shrimp at the polluted site is therefore consistent with a reduced top-down control, caused by reduced predatory capabilities of their major predator mummichogs, which in addition were both smaller and less abundant at the contaminated site (Weis and Candelmo, 2012).

Weis and co-workers not only studied the mummichogs and their prey grass shrimps but also their predator Bluefish, a piscivores fish species. Bluefish from a contaminated site were significantly smaller, had elevated levels of contaminants, and many (73%) of the fish had empty stomachs (8-37% is a normal range) while their prey species were readily available. It is especially interesting to see that the levels of PCB and DDT in prey species found in their stomachs were higher compared to the levels in these prey species caught in trawls and seines. This supports laboratory data that contaminated mummichogs were impaired in predator avoidance and were easier for blue crabs to capture. In another study Bluefish were fed prey species from contaminated or reference sites for four months after which exposed Bluefish displayed significantly reduced feeding, spontaneous activity, swimming speed and growth. The reduced feeding and growth support the data on size and empty stomachs seen in the field-collected fish.

In conclusion, this extensive research demonstrated that altered behavior (reduced feeding/prey capture as well as impaired predator avoidance ability) affected both quantity and quality of the food intake and resulted in reduced growth, while underlying changes in the thyroid glands have been seen in both fish species. Besides these consequences on the population level, community level consequences were also observed in the population dynamics of grass shrimps probably caused by a reduced top-down control. Still, as concluded in the review by Sloman and McNeil (2012), the ecological relevance of these studies should carefully be considered as the effect of simple changes in activity on survival in the natural environment could not be generalized across studies.

Birds, several behavioral responses; feeding and reproductive output

As fish-eating birds are at particular risk for elevated MeHg exposure, Depew *et al.* (2012) surveyed the available literature to summarize the effects of dietary MeHg on the common loon (*Gavia immer*) and to derive ecologically relevant toxic thresholds for dietary exposure to MeHg in fish prey. By combining data from ecotoxicity studies in the laboratory (causal relationships for several parameters such as behavior and growth) and observations on field exposed populations they also provide some insight into the ecological relevance of behavioral effects. The authors proposed three screening benchmarks of 0.1, 0.18, and 0.4 µg MeHg/g wet weight in prey fish. The lowest benchmark (0.1 µg MeHg/g wet weight) is the threshold for adverse behavioral impacts in adult loons and is close to the empirically determined no observed adverse effects level for subclinical effects observed in captive loon chicks. The remaining benchmarks (0.18 and 0.4 µg MeHg/g wet weight) correspond to MeHg levels in prey fish associated with significant reproductive impairment and reproductive failure in wild adult loons.

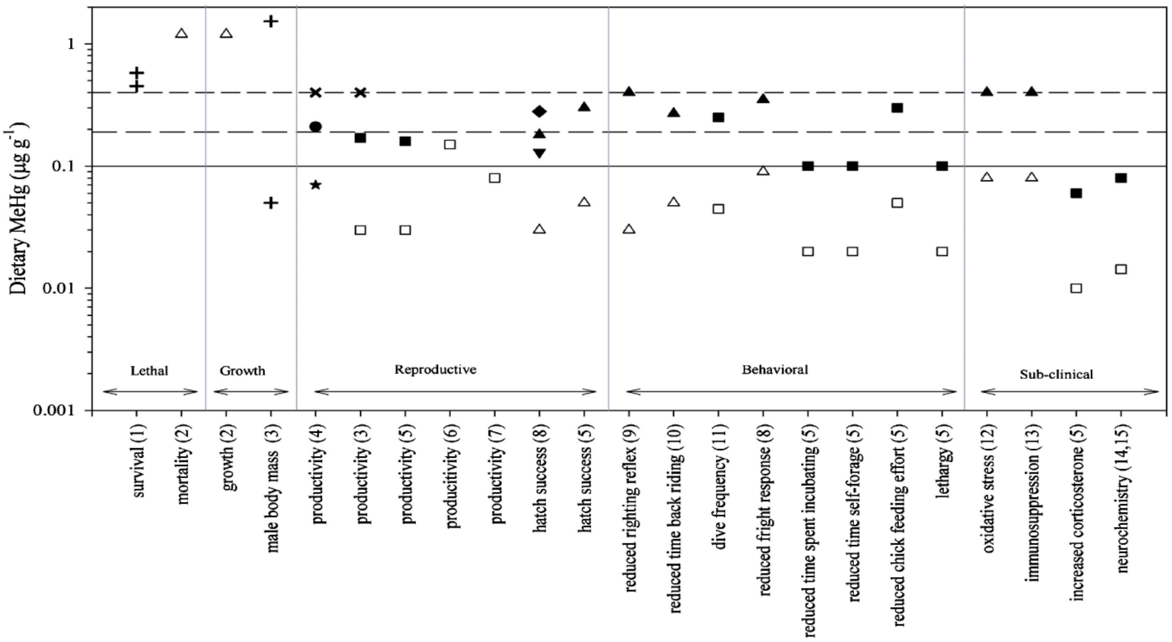

Figure 3.1. Plot of relevant endpoints for deriving screening benchmarks for the common loon copied from Depew *et al.* (2012):

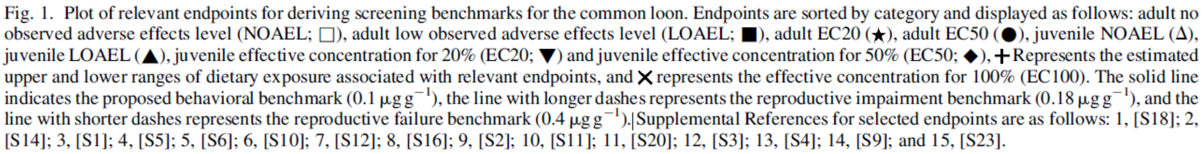
Fig. 1. Plot of relevant endpoints for deriving screening benchmarks for the common loon. Endpoints are sorted by category and displayed as follows: adult no observed adverse effects level (NOAEL; □), adult low observed adverse effects level (LOAEL; ■), adult EC20 (★), adult EC50 (●), juvenile NOAEL (Δ), juvenile LOAEL (▲), juvenile effective concentration for 20% (EC20; ▼) and juvenile effective concentration for 50% (EC50; ◆), + Represents the estimated upper and lower ranges of dietary exposure associated with relevant endpoints, and × represents the effective concentration for 100% (EC100). The solid line indicates the proposed behavioral benchmark (0.1 $\mu g\, g^{-1}$), the line with longer dashes represents the reproductive impairment benchmark (0.18 $\mu g\, g^{-1}$), and the line with shorter dashes represents the reproductive failure benchmark (0.4 $\mu g\, g^{-1}$).|Supplemental References for selected endpoints are as follows: 1, [S18]; 2, [S14]; 3, [S1]; 4, [S5]; 5, [S6]; 6, [S10]; 7, [S12]; 8, [S16]; 9, [S2]; 10, [S11]; 11, [S20]; 12, [S3]; 13, [S4]; 14, [S9]; and 15, [S23].

Depew *et al.* (2012) further illustrated that increased mercury exposure correlates with several changes in behavior of both adult birds and loon chicks, such as an increased frequency of lethargic behavior in breeding adults, more time preening and resting and less time foraging for themselves and significantly reduced amounts of time spent incubating eggs (prey fish containing 0.05-0.15 µg MeHg/g wet weight). In territories where prey fish Hg concentrations exceeded 0.3 µg MeHg/g wet weight, food provisioning for chicks was reduced by 42% relative to that in less contaminated locations (<0.05 µg MeHg/g wet weight). Furthermore, several field studies have documented subtle behavioral alterations in loon chicks at different stages of development. Loon chicks <12d old spent less time back-riding, potentially increasing unnecessary energy expenditures and increasing vulnerability to predation and exposure, while a negative relationship was also observed between the frequency of wing flaps and diving, and a corresponding increase in swimming, peering, and begging as blood Hg concentrations increased in >40-d-old chicks.

It is not hard to hypothesize that at least some of these behavioral impairments may adversely affect productivity of the reproduction. A reduction in time spent incubating eggs will increase the risk of egg loss to nest predation and chilling, while reductions in chick feeding effort may directly impair the growth and survival of loon chicks. However, Depew *et al.* (2012) also concluded that the degree to which these behavioral changes will affect population dynamics is presently unknown. Therefore, the suitability of this benchmark for ecological risk assessment remains limited as long as there is no quantitative relationship between observed behavioral impairments and changes in individual fitness, survival, or reproductive success (Depew *et al.*, 2012). On the other hand the difference between the screening benchmarks for behavioral impacts and significant reproductive impairment is only a factor of 2, and there is a wealth of evidence qualitatively supporting the expected relation between behavioral impairments and productivity. Effect on the behavior of loons as established in the laboratory or the field are therefore still a valuable source of supporting information.

Vertebrates and EDC's

Both Clotfelter *et al.* (2004) and Zala and Penn (2004) reviewed published literature and presented an extensive overview of studies showing adverse effects of endocrine disruptors on cognition and behavior of vertebrates, including correlational and experimental evidence for fish, amphibians, birds and mammals. Based on their review Zala and Penn (2004) concluded that "EDCs have adverse effects on a wide range of behaviors, including sexual and other reproductive behaviors, activity, motivation, communication, aggression, dominance and other social behaviors, and learning and other cognitive abilities". It is easy to hypothesize that these behavioral effects of EDC's will affect population sustainability and indeed, Clotfelter *et al.* (2004) as well as Zala and Penn (2004) mentioned several examples linking behavioral impairments to population sustainability (see both reviews for mentioned references). These examples are often based on field observations and as such correlational of nature. For example, tree swallows living in PCB-contaminated areas build smaller and lower-quality nests and were more likely to abandon or bury their eggs than did birds living in cleaner areas (McCarthy & Secord, 1999a,b). However, some of their examples also contained more causative elements such as

i) DDT exposure in gulls. "In the 1970s, researchers found homosexual pairing between female Western gulls, *Larus occidentalis*, in California (Hunt, 1977). Later work indicated that these birds had high levels of DDT in their eggs, and experimentally injecting DDT into eggs at the levels found in the contaminated gulls' eggs in the 1970s resulted in abnormal sexual development, feminization and even intersexuality of male birds, which caused breeding failure (Fry & Toone 1981)".

ii) Methoxychlor exposure in amphibians. "Exposing salamander embryos, *Ambystoma macrodactylum*, to methoxychlor reduced their startle response and the distance travelled in response after startled. The mechanism underlying the response is unclear (e.g. it may be caused by reduced overall activity or hearing loss), but exposure to this insecticide increased the susceptibility of this amphibian to predation from dragonflies (Verrell, 2000)". Zala and Penn (2004) concluded that "the consequences of chemical-induced changes in behavior are generally unclear, although EDCs and other chemical pollutants appear to be contributing to the decline of some vertebrate populations, such as Florida panthers (Facemire *et al.*, 1995), marine mammals (De Guise *et al.*, 1995) and amphibians (Dalton 2002; Renner 2002)".

Fish, other wildlife and pharmaceuticals

Brodin *et al.* (2014) published a review on the effects of pharmaceuticals on fish behavior and discussed the potential ecological effects. Twenty two studies were found in which the effects of antidepressants, antiepileptic drugs, antihistamines, beta blockers and psychiatric drugs were studied on a diversity of behaviors such as feeding, activity, aggression, boldness and reproductive behavior. Brodin *et al.* (2014) stated that effects of pharmaceutical on behavior are of direct ecological importance, as behaviors are tightly linked to individual fitness and population persistence. However, they also concluded that "despite the potential impact of pharmaceuticals on wildlife behavior, and the demonstrated importance of animal behavior for fitness, population dynamics and ecosystem functioning, few studies have investigated the ecological implications of pharmaceutically induced behavioral modifications" (although 3 examples are cited by Brodin *et al.*, 2014). In addition to their review Brodin *et al.* (2014) also performed an additional experimental study in which both uptake and behavioral impact of the psychiatric drug oxazepam on a predatory fish (the perch, *Perca fluviatilis*) and its invertebrate prey (the damselfly, *Coenagrion hastulatum*) was assessed. The authors demonstrated that perch became more active while damselfly behavior was unaffected, illustrating that behavioral effects of pharmaceuticals can differ between species and that, as a consequence, ecosystem-scale effects are again probable. Unfortunately, the predation risk itself was not studied.

In the same Theme Issue of the Philosophical Transactions, Arnold *et al.* (2014) also published a review on the effects of pharmaceuticals with a widener approach focusing on all vertebrate wildlife encompassing terrestrial, freshwater and marine ecosystems. They for example reviewed research by Säfholm *et al.* on the effects of estrogens and other synthetic hormones which can impair the reproductive functions of amphibians, for example, through their effects on vitellogenesis and reproductive behavior; changes in the behavior of wild starlings exposed to endocrine-active substances and how alterations in aquatic food chains might occur through a behavioral modification in predators and prey exposed to psychoactive medication. As vertebrates, excluding fish, were not the main scope of the present research, the original articles mentioned by Arnold *et al.* (2014) were not included in the present project. However, these reviews by Arnold *et al.* and Brodin *et al.* do illustrate that the question "whether behavioral parameters should be included in environmental risk assessments?" might be particularly relevant for pharmaceuticals as many of them are intended as modifiers of physiology and, in some cases (e.g. psychoactive drugs) also behavior.

Fish; feeding; growth

Kasumyan (2001) reviewed the available literature on the effects of pollutants on the foraging behavior of fish. The author concluded that the sensory organs (olfaction, taste and lateral line) were more sensitive to toxicants compared to the overall feeding rate and that the feeding rate decreased due to suppression of the motivation for feeding and to disturbance of the proper feeding behavior (i.e., food search, distance and speed of reaction to prey, pursuing and catching of the prey, and estimation of its suitability as food). Furthermore the author stated that in environments were feeding behavior was affected, the fish manifest a decrease in growth rate while their fatness, fecundity, and resistance to many environmental factors might also decrease. The feeding intensity and growth rate of fish decreased not only due to insufficient foraging motivation and anorexia, but also due to disturbances of proper foraging behavior, ability to perform the search for food, pursue or grasp the prey, and estimate its acceptability. These elements of foraging behavior were found to be more sensitive to pollutants and develop at lower, sublethal concentrations of toxicants (Kasumyan, 2001). Disturbance of foraging behavior, decrease in the distance and speed of reaction to prey, and of the efficiency of aimed strikes at the prey make food organisms less available to fish and increase energy expenditures related to feeding.

In another review on fish Scott and Sloman (2004) focused on the disruption of behaviors associated with foraging, predator avoidance, reproduction, and social hierarchies as these may be more environmentally relevant than simple behavioral responses to toxicants when considering potential impacts on fish populations. They discussed whether physiological and behavior disruptions can causally be linked. In addition, they reviewed available literature to link these interrelated changes in behavior and physiology with population level consequences but concluded that only very few studies have tried.

Insects and metals

The effects of metal and metalloid pollution on insect behaviors in both terrestrial and aquatic systems in reviewed by Mogren and Trumble (2010). They grouped behavioral parameters into three types (ingestion, taxis, and oviposition) and prepared a qualitative overview of all experimental results including metal, species name and direction of the behavioral effect. For both the terrestrial and aquatic ecosystem most of the observed effects were negative, although positive effects were also noted (with a higher frequency in aquatic environments than in terrestrial studies). Unfortunately the authors didn't discuss on the question if behavioral impairments can be extrapolated to effects on individual fitness or population level. However, based on the titles in their list of references, some of the reviewed studies might contain information on the ecological relevance of the behavior studied, such as "Effects of dietary zinc sulfate on the growth and feeding behavior of *Ostrinia nubilalis*", "Influence of heavy metal ions (Cadmium and Zinc) on the mortality, feeding behavior and reproductive potential of *Neochetina bruchi*" or "Effects of dietary ZnSO4 on the growth and feeding of the tobacco budworm, *Heliothis virescens*". Most probably, these references belong to individual studies (comparable to the examples mentioned in §2.3) and therefore less suitable for more general conclusions.

3.3 Ecological evidence and mathematical models

The ecological relevance of behavior impairments caused by toxicants can also be validated using ecological knowledge. For example, when ecologist have established a relation between decreased food intake (for example caused by reduced food availability) and decreased growth and reproduction, it can be hypothesized that a comparable relation also exist when the decreased food intake is caused by a toxicant. Furthermore, ecological evidence can also be found in existing population models, which for example simulates the life-history of individuals at various environmental conditions. Some examples of both are illustrated below.

Amphibian; movement; growth and predation

The AChE inhibitor carbaryl caused a significant reduction in general activity and sprint speed of leopard frog tadpoles (*Rana blairi*) within 24h of exposure (Bridges, 1997). The ecological relevance can be substantiated in relation to both growth and predation risks. Key-element is an ecological study, demonstrating that the time unstressed tadpoles spend swimming is correlated with time spent feeding (Horat and Semlitsch, 1994) and as such with reduced growth rates. In addition, ecological research also demonstrated that amphibian adult fitness (e.g., survival to first reproduction, fecundity) is correlated with the length of the larval period and the size at metamorphosis (Semlitsch *et al.*, 1988).

Besides these consequences for larval growth and developmental rate, these behavioral impairments also affect the risk of predation both directly and indirectly. First, many tadpoles escape predators by being too large to capture or by emerging from ponds as early as possible. Tadpoles must reach a minimum size before metamorphosis, and fast growth shortens the larval period, thus decreasing exposure to predators (Wilbur *et al.*, 1983). As such reduced growth can lead to indirect mortality by prolonging susceptibility to predators. On the other hand, direct predation risks can both decrease and increase. A decrease in tadpole activity can result in lower predation rates through reduced detection by visually oriented predators (Lawler, 1989; Azevedo-Ramos *et al.*, 1992), while predation rates can also increase if there is a decrease in swimming performance and predator avoidance (Huey, 1980; cited in Bridges, 1997).

Rotifer; movement; predation

Janssen *et al.* (1994) as well as Charoy and Janssen (1999) demonstrated changes in the swimming behavior when the rotifer *Brachionus calyciflorus* was exposed to sublethal copper, PCP or lindane concentrations. During the experiments both speed and duration of swimming was affected. In their discussion the authors turned to ecological literature demonstrating that predation pressure is determined, among other factors, by the swimming behavior of both prey and predator to underpin the ecological relevance of swimming behavior. For example, Greene (1986; 1988) studied prey-selection patterns of copepods in detail and stated among others that "a reduced activity often provides fewer visual cues for active hunters to locate their prey, important for eliciting a predator attack". Meanwhile, this expected relation between toxicants, swimming behavior and risk of predation has also been tested in multispecies experiments (Gomez *et al.*, 1997; Preston *et al.*, 1999). Both articles indeed demonstrate an interaction between toxicants, swimming behavior and risk of predation. The patterns were however not always consistent with each other. Gomez *et al.* (1997) for example demonstrated a reduced capture and ingestion efficiency for the predatory rotifer *Asplanchna girodi*, indicating that this species was more susceptible for the sublethal effects of PCP than its prey species *Brachionus calyciflorus*. In addition, Preston *et al.* (1999) studied the effects of PCP on the vulnerability of several rotifer species to predation by *A. girodi* and demonstrated that, due to differential responses of prey species to PCP, changes in prey vulnerability after exposure varied among prey species. Changes in the swimming behavior of rotifer prey species might therefore generate both an increased as well as a decreased risk of predation, depending on the susceptibility of both prey and predator, the hunting strategy, the size of prey and predator and probably numerous other factors such as the simultaneous presence of other prey species. On the other hand, the interactions as observed in the multispecies experiments does indicate the ecological relevance.

Amphipods, feeding, growth and reproduction

Several authors studied feeding behavior in exposed amphipods. For example, both Alonso *et al.* (2009) and Felten *et al.* (2008) found decreased feeding rates in cadmium-exposed *Gammarus pulex*; Kalcikova *et al.* (2014) studied predation on mayfly nymphs and leaf consumption in *Gammarus fossarum*, which both decreased under a simultaneous exposure to TiO_2-nanoparticles and UV-light and Agostinho *et al.* (2012) and Pestana *et al.* (2007) demonstrated decreasing feeding rates in the amphipod *Echinogammarus meridionalis* when exposed to cadmium, zinc or copper.

In several of these example feeding behavior of amphipods was assessed using "leaf litter breakdown" (e.g. Kalcikova *et al.*, 2014; Zubrod *et al.*, 2014). This automatically implies effects on decomposition rate, a fundamental ecosystem function. In a stream impacted by point-source discharges, a strong positive correlation was for example observed between *G. pulex* feeding rate measured in situ and total leaf decomposition measured at the same site, suggesting that the in situ feeding assay can be used as an indicator of this important ecosystem process (Maltby *et al.*, 2002).

Feeding rates can also affect the fitness of amphipods themselves. The relation between energy-intake (i.e. feeding rate) and growth forms for example the base for the "scope for growth" theory. Scope for growth (SfG) is a measure of the energy balance of an animal (i.e., the difference between energy intake and metabolic output). In a review Maltby (1994) concluded that exposure to a range of toxicants resulted in decreases in *Gammarus* SfG, which were qualitatively and quantitatively correlated with subsequent reductions in growth and reproduction (offspring size and brood viability). Reductions in feeding rates were also correlated with changes in community function (i.e. leaf processing) and may be indicative of changes in community structure. In fact, Maltby and co-workers (Maltby *et al.*, 2002) showed that a reduction of *G. pulex* feeding rates was associated to a reduction of its abundance and also with a reduction of detritus processing and macroinvertebrate diversity in contaminated environments (see figure 3.2). According to Maltby (1994), stress-induced changes in *Gammarus* energetics can therefore be linked, in a mechanistic way, to effects at higher levels of biological organization. Furthermore, Maltby (1994) concluded that these reductions in SfG were primarily determined by reductions in energy intake, and as such measuring SfG might be simplified to a measure of feeding rate.

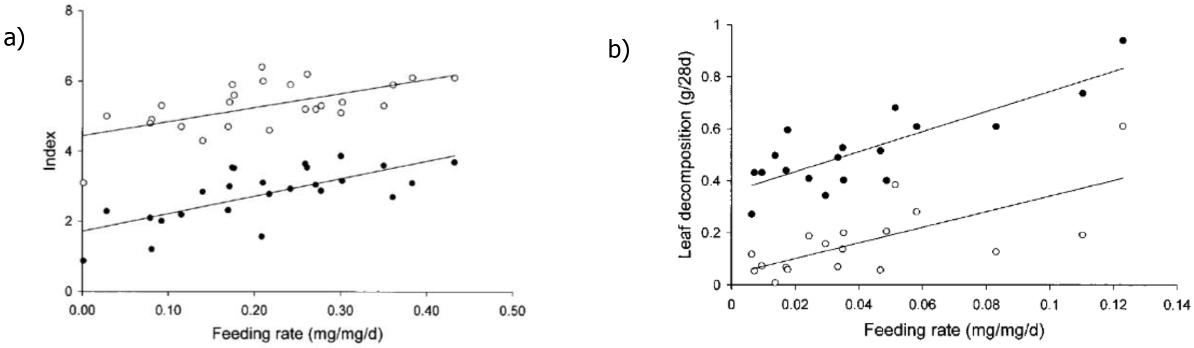

Figure 3.2 Correlation between *in-situ* feeding-rates of *Gammarus pulex* and a) Shannon diversity (closed symbols) and ASPT-index (open symbols) and b) leaf decomposition at reference and contaminated sites. Figures copied from Maltby *et al.*, 2002.

Furthermore, the ecological relevance of decreased feeding rates in amphipods is also demonstrated by Moore and Farrar (1996; cited in Felten *et al.*, 2008), who showed that growth rates and reproduction of *H. azteca* decrease significantly with reduced food rations. Coulaud et al. (2015) performed comparable experiments in which they focused on the impacts of food deprivation on reproductive endpoints in *Gammarus fossarum*. They found that food deprivation triggers a slowdown of the moulting process and a reduction in fertility, but no alteration on embryonic development. Furthermore they concluded that these reproductive impairments already appeared at food deprivation values usually recorded in monitoring programs of environmental pollution (using the *in situ* feeding bioassay). Consequently, decreasing feeding rates may influence the population as a whole.

In addition to the amphipod studies discussed above, scope for growth is frequently studied for several bivalve and fish species (e.g. Wong and Cheung, 2001). This literature is not reviewed at present, but suggests that with a comparable approach behavioral impairments on the feeding rate of bivalves or fish can be linked with ecological relevance as well (e.g. Riisgard *et al.*, 2013 showing a relation between feeding and growth in *Mytilus edulis*). The same might apply to other species such as mysids (Verslycke *et al.*, 2004) and sea urchins (Stumpp *et al.*, 2011) for which SfG has also been used as an indicator of toxicant stress.

Fish; feeding; population growth (modelling)

Baldwin *et al.* (2009) evaluated how the sublethal impacts of pesticides on physiology and behavior can reduce the somatic growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*) and, by extension, subsequent size dependent survival when animals migrate to the ocean and overwinter in their first year. They based their modelling on a previous published empirical relationship between AChE activity and the feeding behavior of juvenile salmon, an already established association between feeding rate and juvenile growth and the assumption (based on available literature) that for several insecticides the time to effect was within a few days. The resulting distributions of individual masses for salmon were used to estimate the size-dependent survival rates for subyearling salmon as well as the consequences for entire populations. The key finding form their work was that demographic changes in salmon populations can be quantitatively extrapolated from pesticide effects on individuals. The model outputs show that environmentally realistic pesticide exposures may limit the recovery potential of salmon populations via delayed reductions in growth and survival as a consequence of behavioral impairments in feeding caused by AChE-activity reductions.

Fish; predation; population dynamics (modelling)

Murphy *et al.* (2008) used previously published results of a laboratory study on the effects of MeHg on larval croaker swimming speed and predator-evasion skills (Alvarez *et al.*, 2006) and combined these with an individual based model to simulate the effects on larval survival and growth. In the laboratory study MeHg-contaminated food was fed to adult female croaker, who were then spawned and their eggs collected. Eggs from the control, low and high MeHg dose treatments were hatched in the laboratory and resulting larvae were evaluated for their growth rates and survival skills. Murphy *et al.* (2008) performed simulations to separate the effects of MeHg exposure on larval encounters with their zooplankton prey (growth only) versus MeHg effects on larval encounters with their predators (mortality only). Laboratory results were analysed with a regression tree to obtain the probability of control and MeHg-exposed larvae escaping a real predatory attack. Measured changes in swimming speeds and regression tree-predicted escape abilities induced by MeHg exposure were then inputted into an individual-based larval fish cohort model. The individual-based model predicted larval-stage growth and survival under two alternative predator composition scenarios (medusa-dominated and predatory fish-dominated). Under MeHg exposure, stage survival was 7–19% of control survival, and the roughly 33-day stage duration was extended by about 1–4 days. MeHg effects on larval growth dominated the response under the medusa-dominated predator composition, while predation played a more important role under the fish-dominated predator composition. By combining these experimental results on swimming speed and predator-evasion skills with individual based modelling, Murphy *et al.* (2008) provided additional reasoning to support the hypothesis that laboratory observed behavioral impairments in predator-prey relationships can indeed have consequences for population sustainability.

Daphnids; feeding; population growth (modelling)

Feeding behavior has been recognized as an ecotoxicological endpoint in filter feeders, because it has a physiological implementation closely associated with growth, metabolism and reproduction (Day *et al.*, 1987). Numerous authors have successfully studied toxic effects on feeding behavior in laboratory or field circumstances (e.g. McWilliam and Baird, 2002 a,b and references in table 2.2). However, some also encountered difficulties in the interpretation due to for example the presence of an interrupted dose-response curve or the absence of an effect at longer exposure periods (Gokcen, 1998).

In a few studies relations with individual or population fitness were discussed. Agatz *et al.* (2013) for example performed experiments with imidacloprid to quantify effects on feeding behavior, mortality, growth and reproduction and did so with different food densities. The ecological relevance of feeding behavior was tested using the individual *Daphnia magna* population model IDamP as a virtual laboratory. IDamP is an individual based population model for *D. magna*, which simulates the life-history of individual daphnids and their plasticity at various environmental conditions including various feeding scenarios. Agatz *et al.* concluded that the interpretation of experimental results should take the effect-cascade, and thus the ecology of the test species into account as the consideration of individual end points would have led to a different interpretation. While effects on survival, growth and reproduction are normally considered as direct toxicity, Agatz *et al.* demonstrated that all these effects (including hormesis) could be explained by hypothesizing that only feeding was affected by imidacloprid, and that this in turn caused the other impacts on individual performance. The authors state that their conclusions should be verified with other toxicants and preferably other organisms. This is especially relevant since *Daphnia* is known to be relatively insensitive to the toxic effects of imidacloprid (Smit, 2014) when considering "traditional" endpoints. Which raises the question whether *Daphnia* would have been considered more sensitive if feeding-rate would have been included in setting an EQS-value. The study by Agatz *et al.* (2013) illustrates however that this question is not easy to answer as these interactions led to different results. For example, under food limitation, the lowest imidacloprid concentration tested (0.15 mg/l) showed a growth-discontinuation, whereas organisms at high food density showed no growth-discontinuation even when exposed to 12 mg/l. The feeding assay showed that a concentration of 12 mg/l reduced the feeding rate by 97%, whereas a concentration of 0.15 mg/l reduced the amount of food eaten by only 3.7%. Hence, Agatz *et al.* concluded that delayed effects on growth occurred under food limitation after short-term feeding inhibition of less than 5% and that differences in the energy reserves caused by the difference in food density might have evoked this behavior. Still, Smit (2014) mentioned a lowest, chronic NOEC-value of 1.768 mg/l (number of neonates), while Agatz *et al.* (2013) stated that an imidacloprid concentration of 0,19 mg/l caused a 5% reduction in feeding rates when exposed for 24 hours, which is an order of magnitude lower.

Agatz *et al.* (2013) supported their reasoning with ecological research on the energy availability and feedings rates in daphnids (see references in Agatz *et al.*, 2013). If their conclusion that only feeding was affected by imidacloprid can be generalized to other organisms and toxicants, it is a strong support for the ecological relevance of feeding behavior.

At the same time the observation that a significant effect on feeding rates did not always cause a comparable effect on the more classical parameters as growth and reproduction, raises an interesting point for discussion. Taking the TGD-EQS (EC, 2011) as starting point, a clear relation between behavioral parameters and population sustainability should be established before these parameters can be used in setting environmental quality criteria. It can be argued that this is not the case as other ecological parameters (e.g. food density) interfered with such a

relation. This is also indicated by other authors, such as Gabsi *et al.* (2014) who used the same mathematical model to demonstrate that several ecological interactions (predation, competition) interfered with the toxic response on population levels. In the absence of a clear relation between behavioral parameters and population sustainability, the use of behavioral effects in setting environmental quality criteria would not be advised (TGD-EQS; EC, 2011).
On the other hand, it can also be argued that the same holds for growth, reproduction and mortality as indicated by Gabsi *et al.* (2014). The discussion should therefore be aimed at the statement in the TGD-EQS (EC, 2011) that either a "definite correlation" or a "causal relationship" should be established. Which statistical certainties are requested for a "definite correlation" and when is a "causal relationship" sufficiently established. In the present case with feeding rates, it can for example easily be argued that feeding is for certain correlated with population sustainability, albeit in the extreme situation that feeding completely ceased. However, several theoretical models such as Scope for Growth (see above), dynamic energy budget theory (DEB; Jager *et al.*, 2014) or bioenergetics models for fish (Sandheinrich and Atchison, 1990) offer other mathematical and mechanistic approaches for linking energy intake, life-history traits of individuals and population level effects. In the case of fish, feeding behavior and predator-prey relations are intermingled. By using their bioenergetic model Sandheinrich and Atchison (1990) for example demonstrated how toxicant effects on components of fishes' predation sequence can modify the size-frequency distribution of prey in the fishes' diet and how reductions in the amount of food consumed may alter growth.

Based on these findings it is concluded that feeding-rate seems to fulfil the requirements set by the TGD-EQS (EC, 2011) for the use of additional parameters in the setting of environmental quality criteria and such use should therefore be considered.

4 Concluding remarks

In the previous chapters several examples were shown, illustrating correlational or causal relations between behavioral impairments and population sustainability. Furthermore, several authors reviewed available literature and discussed on how these results could be used in environmental risk assessments. In §4.1 these findings are summarized to aid a discussion on a more integrated use of behavioral effect parameters in risk assessments, while §4.2 provides some recommendations for future work.

4.1 Should behavioral parameters be used in environmental risk assessments?

As stated in the TGD-EQS (EC, 2011) behavioral parameters can be used in environmental risk assessment when a clear relationship with population sustainability exist, based on either a definite correlation or a causal relationship. While dozens of studies were found demonstrating a correlation between behavioral disruptions and individual fitness (growth, reproduction, survival), studies focusing on the relations with population sustainability or a generalization of these individual studies are more scarce. In answering the question whether behavioral parameters could be used in environmental risk assessments attention should therefore be focused on several underlying questions:

i) Which organisms and types of behavior are most frequently studied and therefore most likely to be the first for which a clear relationship with population sustainability can be established?
ii) Which more generalized conclusions on the relationships with population sustainability have been put forward in existing reviews?
iii) What can be said on the sensitivity of behavioral impairments compared to chronic, sublethal effects on growth and reproduction?
iv) Do field studies support the extrapolation of laboratory observations to field conditions and can these studies substantiate an effect on population sustainability?
v) Can ecological research and/or population modelling provide additional lines of evidence to support the relation between behavior and population sustainability?

Ad i) Which organisms and types of behavior are most frequently studied and therefore most likely to be the first for which a relation with population sustainability can be established?

The overview of available literature presented in chapter two demonstrates that around 50% of the data on behavioral effects belong to mammalian and fish studies, with insects and crustacean as the third and fourth most frequently studied group of organisms. Most of the behavioral activities relates to food consumption and feeding (effect parameters such as feeding efficiency, filtration rate, predator behavior) or movement (e.g. swimming, predator avoidance, distance moved, burrowing).

As mammals were outside the scope of the present study, the question is focused on "whether sufficient evidence exist to consider the use of toxicity information for two clusters of behavior (feeding and movement) for three groups of organisms (fish, insects and crustaceans).

Ad ii) Which more generalized conclusions on the relationships with population sustainability have been put forward in existing reviews?

In a review Amiard-Triquet (2009) concluded that the higher sensitivity of behavioral disturbances compared to acute lethality has been clearly established, while on the other hand behavioral endpoints are not more sensitive than the more classical sublethal biomarkers of pollution, such as several biochemical and physiological changes. In addition, Amiart-Triqu*et al*so reviewed several articles demonstrating a good agreement between behavioral impairments in experimental and field studies as well as several publications highlighting the ecological relevance of behavioral impairments (which is not always the same as an proven effect of population sustainability). These last examples related primarily to predator-prey studies although Amiard-Trigu*et al*so referred to another review by Jones and Reynolds (1997) in which a few studies were discussed linking reproductive behavior to reproductive success and one study dealing with effects on populations. In addition, Preston (2002) reviewed the scientific literature on the indirect effects of toxicants and summarized several articles on predator-prey-relations. His review showed that existing examples are sufficient to qualitatively illustrate a relation between behavioral responses in activity or movement and individual fitness or population level effects. At the same time, Preston (2002) also stated that so far little attempt has been made to quantitatively assess the impacts at higher levels of biological organization.

In their review on endocrine disrupting compounds (EDC's) Zala and Penn (2004) concluded that "EDCs have adverse effects on a wide range of behaviors, including sexual and other reproductive behaviors, activity, motivation, communication, aggression, dominance and other social behaviors, and learning and other cognitive abilities". The ecological relevance of the behavioral impairments was illustrated with several examples based on field studies and as such correlational of nature. However, some of their examples also contained more causative elements such as DDT exposure in gulls and methoxychlor exposure in amphibians.

Together these reviews illustrate that a good agreement between behavioral impairments in experimental and field studies is often found and that a more limited number of studies demonstrated a correlational relation between behavioral impairments and population sustainability. According to the EC (2011) this might be sufficient to include effects on these behavioral parameters and organisms in future risk assessments. However, this still leaves questions unanswered such as: Do these examples form a sufficient burden of evidence resp. can these examples be extrapolated to other species, contaminants and behaviors? Should each disruption in behavior be considered as a negative effect even when these might fall within the natural fluctuations resp. how to quantitatively assess the impacts at higher levels of biological organization?

Ad iii) What can be said on the sensitivity of behavioral impairments compared to chronic, sublethal effects on growth and reproduction?

Although exceptions exist, individual studies often point out that concentrations at which acute effects on behavior occur (within hours or days), are more or less comparable to chronic effect levels for growth or reproduction. To substantiate this impression based on individual studies, Melvin and Wilson (2013; §2.3) performed a meta-analysis for fish and crustacean in which the relative sensitivities of behavioral studies were compared to those assessing acute lethality, development and reproduction. They concluded that overall behavioral studies were more sensitive than those assessing developmental and reproductive endpoints. For studies with crustaceans as the model organism only, there was however no statistical difference in the sensitivity of behavioral, developmental or reproductive studies. Conversely, behavioral responses had greater sensitivity than those investigating developmental or reproductive endpoints in studies with fish as the model organism. Although being statistical significant, the 95% confidence intervals showed a large overlap and the differences were quantitatively not extreme (see figure 2.2). It can therefore be concluded that for these taxa the sensitivity of acute behavioral responses is in orders of magnitude comparable with chronic effects on growth and reproduction.

As a definite correlation is sufficient according to the TGD-EQS (EC, 2011), it might be stated that an experiment studying only behavioral responses for fish or crustacean could be included in a risk assessment without further supporting evidence for population level effects. In such a case the follow-up question will be the choice of appropriate assessment factors (see below).

Ad iv) Do field studies support the extrapolation of laboratory observations to field conditions and can these studies substantiate an effect on population sustainability?

Two examples were found illustrating that behavioral impairments as observed in the laboratory indeed do occur under field conditions, and thereby influence population sustainability or even communities.

a) Extensive research on behavioral responses, predator/prey relationships and growth of two fish species has been carried out by Weis and co-workers as reviewed by Weis and Candelmo (2012; §3.2). They combined laboratory studies on feeding behavior and toxic effects with field observations on the mummichog *Fundulus heteroclitus* and his predator the bluefish *Pomatomus saltatrix* living in contaminated estuaries to examine population and community level consequences. This extensive research demonstrated that altered behavior (reduced feeding/prey capture as well as impaired predator avoidance ability) affected both the quantity and quality of the food intake and resulted in reduced growth and increased predation risks under field conditions. Besides these population level effects, community level consequences were also observed in the population dynamics of grass shrimps probably caused by a reduced top-down control.

b) Maltby and co-workers frequently studied feeding behavior of *Gammarus* in conjunction with the Scope for Growth theory. In a review Maltby (1994; §3.3) concluded that exposure to a range of toxicants resulted in decreases in *Gammarus* Scope for Growth, which were qualitatively and quantitatively correlated with subsequent reductions in growth and reproduction. Reductions in feeding rates were also correlated with changes in community function (i.e. leaf processing). In fact, Maltby and co-workers (Maltby *et al*., 2002) showed that a reduction of *G. pulex* feeding rates was associated with a reduction of its abundance and also with a reduction of detritus processing and macroinvertebrate diversity in contaminated environments. According to Maltby (1994), stress-induced changes in *Gammarus* energetics can therefore be linked, in a mechanistic way, to effects at higher levels of biological organization.

These field studies provide correlational evidence on the relationship with population sustainability. More of these studies will therefore be valuable, to support an extrapolation of their conclusions to other species, circumstances and toxicants. Again, one of the remaining questions is whether these examples provide a sufficient burden of evidence to sustain the use of behavioral parameters in risk assessments and if so, with which Assessment Factors.

Ad v) Can ecological research and/or population modelling provide additional lines of evidence to support the relation between behavior and population sustainability?

From way back ecologist have been studying the behavior of animals in relation to their individual fitness or population sustainability. Ecological literature is therefore a very useful source of evidence demonstrating the ecological relevance of behavior. In the present review some individual examples are mentioned (§3.3). However, the literature also offers possibilities for a more general approach. Several theoretical models such as Scope for Growth, the dynamic energy budget theory (DEB) and bioenergetics or population models for fish offer mathematical and mechanistic approaches for linking energy intake, life-history traits of individuals and population level effects. By using their bioenergetic model Sandheinrich and Atchison (1990) for example demonstrated how toxicants can modify the size-frequency distribution of prey in the fishes diet and how reductions in the amount of food consumed may alter growth. In addition to these models focusing on feeding and growth, Murphy *et al*. (2008) combined experimental results on swimming speed and predator-evasion skills of fish larvae with individual based modelling and provided additional reasoning to support the hypothesis that also laboratory observed behavioral impairments in predator-prey relationships can have consequences for population sustainability.

The present literature review, with an exploratory character, did not intend to provide a complete overview. Still, it is considered useful to summarize the examples discussed in the previous chapters creating an overview of the types of behavior (movement, feeding or other/not specified) for which plausible evidence exist on its ecological relevance (table 4.1). This table illustrates that the ecological relevance of behavioral impairments is primarily studied for two types of behavior, "movement" (e.g. predator avoidance, burrowing behavior, swimming speed) and "feeding" (e.g. feeding rates, filtration, leaf litter breakdown). As summarized above, several lines of evidence exist for both types of behavior substantiating each other. The ecological relevance of behavioral parameters belonging to the group "movement" is mostly related to predation risks, while feeding behavior is relevant for energy budgets and as such for growth and reproduction.

Table 4.1. Overview of the types of behavior (movement, feeding or other/not specified) for which plausible evidence exist on its ecological relevance. The lines of reasoning are categorized into four groups; meta-analysis, multi-species tests, case studies and ecological evidence.
A=Meta-analysis (§2.3); B=Multi-species tests (§3.1); C=Case-studies combining laboratory and field data (§3.2); D=Ecological evidence and mathematical models (§3.3)

	Movement	Feeding	Other or not specified
Birds	-	-	feeding & reproduction (C)
Fish	predation (B) growth & predation (C,D)	growth (C,D)	various behavior vs. growth & reproduction (A)
Amphibian	growth (B) growth & predation (D)	-	-
Insects	predation (B)	-	-
Crustacean	predation (B)	population growth (D) algal growth (B)	various behavior vs. growth & reproduction (A)
Amphipods	-	growth & reproduction (D) community effects (C)	-
Rotifer	predation (B,D)	-	-

While each individual line of reasoning provides interesting examples and supporting evidence, they also leave several questions unanswered (see above). However, by combining these individual lines of reasoning in a weight of evidence approach, it might be concluded that the question whether "behavioral effects should be incorporated in environmental risk analyses" might be answered affirmative as long as the behavioral parameter studied belong to "movement" or "feeding" and the organisms are either fish or crustacean. Within these groups i) laboratory studies demonstrated a relation with individual fitness (growth, reproduction); ii) a meta-analysis verified that behavioral parameters were comparable to or somewhat more sensitive than growth and reproduction; iii) several studies demonstrated comparable behavioral impairments under field conditions while iv) some also demonstrated a correlation with population and even community level consequences and v) ecological theory and population models are in support.

However, several questions still need further research such as i) It is not yet said that each disruption in behavior should be considered as a negative effect as some might fall within the natural fluctuations; ii) More studies might be required to generalize these findings to other circumstances, toxicants and species and iii) If behavior is used in risk assessments, which assessment factors should be used?

As always, uncertainties can be dealt with in future studies (§4.2). Furthermore, the TGD-EQS (EC, 2011) mentions that in establishing the size of assessment factors, a number of uncertainties must be addressed to extrapolate from single-species laboratory data to a multi-species ecosystem; i) intra- and inter-laboratory variation of toxicity data; ii) intra- and inter-species variations (biological variance); iii) short-term to long-term toxicity extrapolation and iv) laboratory data to field impact extrapolation. In the case of behavioral parameters, most of these are only seldom being studied (exceptions exist such as Eissa *et al.*, 2010). However, some of the uncertainties addressed for behavioral parameters also apply to growth and reproductive parameters. Differences in food availability, inter- and intra-specific interactions, predation, other stressors as current velocity, availability of habitats etc can for example all affect the extrapolation between laboratory toxicity tests and the effect levels under field conditions (e.g. Kattwinkel and Liess, 2014). At the same time the meta-analysis mentioned above indicated that the sensitivity of short-term behavioral impairments were in orders of magnitude comparable to chronic effects on growth and reproduction and including ecotoxicological information on additional species would only provide a better insight in interspecies variability. As starting point for a further discussion, these arguments support the use of behavioral impairments in a way comparable to chronic effects on growth and reproduction, including the choice of an AF (as long as the behavioral parameter studied belong to "movement" or "feeding" and the organisms are either fish or crustacean).

It should be realized that uncertainties will always remain, whatever future studies will demonstrate. The question "whether sufficient evidence exist to highlight a clear relation between behavioral parameters and population sustainability?" might therefore also be rephrased in "would inclusion of behavioral parameters in environmental risk assessments either increase or decrease the reliability of the Environmental Quality Standards set?". Except for some well-studied toxicants like metals, environmental risk-assessments are often characterized by a poverty of ecotoxicological data, especially chronic NOEC-values. Available evidence (meta-analysis) further demonstrate that the sensitivity of acute behavioral responses was more or less comparable to chronic effects on growth and reproduction, indicating that inclusion of behavioral parameters may not lead to markedly different NOEC-values for species already being present based on growth and reproduction. A strong argument in favor of including behavior is that the addition of these parameters will result in an increased number of species being studied. Based on the present exploratory literature review, it is therefore felt that sufficient evidence is available to start considering NOEC-values for behavioral impairments on movement and feeding for fish and crustacean in the same manner as chronic NOEC's for growth and reproduction. Lowering the AF in the presence of behavioral parameters for additional (fish and crustacean) species should therefore be considered as an option. The reduced uncertainty in risk assessments by including more data and more species is in that case favored above the remaining uncertainties concerning the ecological relevance of behavior.

4.2 Recommendations for future work

Remaining uncertainties and future research

Future research might address several aspects and limitations as mentioned by Amiart-Triquet (2009), such as remaining inconsistencies of a number of observations, the ecological context determining whether or not pollutant-induced behavioral changes will occur in the field or focus on species that have a key role in the structure and functioning of the ecosystems. Inconsistencies might for example be the absence of a clear dose-response curve, the observation that the strength of the behavioral impairment can be reduced at longer exposures or a change in the direction of effect (hyper- versus hypoactivity) with an increasing exposure time (Gokcen, 1998; Fryday *et al.*, 1996; Khalil *et al.*, 2013). It is illustrative that Hart (1993) in his study on starlings concluded that "a review of relevant studies suggests that only broad generalizations, of limited practical value, can be made about the relationship between AChE inhibition and behavioral effects". Furthermore several authors concluded that in addition to the existing, rather qualitative evidence concerning the ecological relevance, little attempt has been made to quantitatively assess impacts at higher levels of biological organization sufficient to be of use to risk assessors (Preston, 2002; Kramer *et al.*, 2011).

Another point of concern are all other kinds of behavior being studied for which the present review did not yet find sufficient evidence to indicate its ecological relevance. For example, the time needed for a snail to right itself after being turned onto its back (Burris *et al.*, 1990), the gravitactic orientation behavior of flagellates (Tahedl and Häder, 2001), the attachment of the freshwater polyp *Hydra* (Quinn *et al.*, 2008), learning acquisition in frogs (Strickler-Shaw and Taylor, 1990) or foot retraction in molluscs (Bringolf *et al.*, 2007) and ventilator activity. Vellinger *et al.* (2013) demonstrated for example that the ventilatory activity of the gammarid *G. pulex* was insensitive to the effects of cadmium and arsenate exposure (while effects did occur on general movement) and concluded that ventilation is reduced as a last resort, to prevent the direct mortality of exposed organisms.

Another parameter to discuss is "avoidance". Avoidance of polluted environments is frequently studied (e.g. De Lange *et al.*, 2006; Dornfeld *et al.*, 2009; Kravitz *et al.*, 1999; Loureiro *et al.*, 2005) and possibly ecologically relevant as it contributes to a reduced exposure. On the other hand, as stated by Amiard-Triquet (2009): It would be imprudent to consider that all living beings have the capability to escape polluted environments. The ecological context is indeed very important in determining whether or not pollutant-induced behavioral changes will occur in the wild. For instance, the lake whitefish *Coregonus clupeaformis* exhibits avoidance of low concentrations of metals under standardised conditions of light but in the case of competing gradients of light and metals, the fish prefer the contaminated shade to the uncontaminated high light intensity, except at the highest concentration tested. Another example is find in the drift response of mayfly nymphs which was increased under cypermethrin exposure but decreased by high stream velocities (the interactions of both factors was significant and antagonistic; Dabrowski *et al.*, 2005). Still, Robinson (2009) mentioned that enough field validation has been conducted for the avoidance response in fish as to accept this parameter as a useful endpoint within a number of pieces of legislation (e.g. site specific and/or weight of evidence approaches under the Natural Resource Damage Assessments [NRDA, 1986], Oil Pollution Act [OPA, 1990] and the Federal Power Act [FPA, 1920]; see overview in Little, 2002, who concluded that the applicability of behavioral measures in environmental regulation increases as the focus becomes increasingly site-specific). Such an use is aided by a further standardisation of test protocols.

Extending the present exploratory literature review
Additional research on the ecological relevance will surely aid the discussion on whether or not behavioral responses can be included in standard setting and risk assessments. At the same time, a more extensive literature search can also prove valuable as the present exploratory overview is not complete. Attention could be focused on behavioral responses outside "movement" and "feeding" and species outside "fish" and "crustacean". For example, the "scope for growth" (SfG) theory is not only applicable for amphipods as feeding behavior (filtration rates) in relation to SfG is also frequently studied for bivalves. This literature is not reviewed at present, but suggests that with a comparable approach behavioral impairments on the feeding rate of bivalves can be linked with ecological relevance as well. An extended literature review might also find other examples in which the population level consequences of behavioral impairments are highlighted for example focusing on different types of reproductive behavior.

Besides extending the scope with other types of behavior as well as other organisms, also several toxicants might require specific attention. Especially pharmaceuticals, as many of them are intended as modifiers of physiology and, in some cases (e.g. psychoactive drugs) also behavior. In such cases behavioral parameters might be much more sensitive compared to traditional ecotoxicological endpoints as survival, growth and reproduction. Several examples of population level consequences of pharmaceuticals have recently been reviewed by Arnold *et al.* (2014), while other merely stress the possible ecological implications of behavioral responses triggered by an exposure to pharmaceutical (Hedgespeth *et al.*, 2014).

A trial and error approach
As for all major changes in an assessment approach, a decision on the role of behavioral parameters in risk assessment might be helped by a more practical "trial and error": Comparing the outcome of risk assessments with and without the behavioral parameters will provide insight in the magnitude of such an effect as well as on the size of the AF which might be applied. In some cases, e.g. pesticides, it will also be possible to compare the outcomes with effects on community levels as observed in mesocosm studies.
The approach followed by Depew *et al.* (2012; see figure 3.1) in which an overview is prepared of all effect parameters being studied and their effect classes is one way to clearly present all available data and can be used as a starting point to discuss the role of behavioral (and other) parameters for example for pharmaceuticals as mentioned above. Instead off starting an environmental risk assessment with eliminating information on 'non-traditional' parameters, all available information is being gathered and presented. In such a case, the EQS can still be based on traditional endpoints and at the same time be compared with additional information to judge whether the EQS is sufficiently protective. Or the behavioral parameters are taken into consideration in the choice of an AF, which decision is than easily supported by such an overview.

These and other considerations are summarised by Robinson (2009) in a review on the use of behavioral effects within ecological risk assessments. His final remark that "*what is perhaps most critical at this point is for risk assessors to begin to use, in regulatory ERAs, the information that currently exists*" underpins the present advise just to start and learn by doing.

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