

1 **Realistic low-doses of two emerging contaminants change size distribution of an annual**  
2 **flowering plant population**

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28  
29 **Abstract**  
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31 HHCB [1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta(g)-2-benzopyran] and 4-*tert*-  
32 octylphenol [4-(1,1,3,3-tetramethylbutyl)phenol] are widely used emerging contaminants that have  
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34 the potential to cause adverse effects in the environment. The purpose of this study was to observe if  
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36 and how environmentally realistic concentrations of these contaminants alter growth in plant  
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38 populations. It was hypothesized that within an exposed *Gypsophila elegans* Bieb (annual baby's  
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40 breath) population especially fast-growing seedlings are impaired even when the population mean is  
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42 unaffected, and small doses can cause hormesis and, thus, an increase in shoot or root length. In a  
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44 dose-response experiment, an experimental population of *G. elegans* was established (total 15.600  
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46 seeds, 50 seeds per replicate, 24 replicates per concentration, 5.2 seedlings/cm<sup>2</sup>) and exposed to 12  
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48 doses of HHCB or 4-*tert*-octylphenol. After five days, shoot and root length values were measured  
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50 and population averages, as well as slow- and fast-growing subpopulations, were compared with  
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52 unexposed controls. Growth responses were predominantly monophasic. HHCB seemed to  
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26 selectively inhibit both root and shoot elongation among slow- and fast-growing individuals, while  
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27 4-*tert*-octylphenol selectively inhibited both root and shoot elongation of mainly fast-growing  
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4 seedlings. The  $ED_{50}$  values (dose causing 50 % inhibition) revealed that the slow-growing seedlings  
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6 were more sensitive and fast-growing seedlings less sensitive than the average of all individuals.  
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9 Although there was toxicant specific variation between the effects, selective toxicity was consistently  
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11 found among both slow- and fast-growing plants starting already at concentrations of  $0.0067 \mu M$ , that  
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13 are usually considered to be harmless. This study indicates that these contaminants can change size  
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15 distribution of a plant population at low concentrations in the  $nM/\mu M$  range.  
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23 **Keywords** – Dose-response, Growth stimulation, Hormesis, Low toxin doses, Selective toxicity.  
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37 **Introduction**

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28 Some specific classes of substances called ‘emerging contaminants’ have been defined to be  
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539 chemicals or materials which cause or have the potential to cause adverse effects on humans and/or  
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740 the environment and, thus, require our special attention (Sauvé and Desrosiers 2014). Usually, these  
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1041 compounds are present in many widely used everyday products such as plastics, flame retardants and  
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1242 cosmetics. They have become a serious environmental issue after being detected in trace  
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1443 concentrations around the globe thanks to the rapid development of analytical techniques enabling  
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1744 identification and quantification of these contaminants (Klaschka et al. 2012; Tao et al. 2011).

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1945 Once being released into the environment, many of these chemicals have been observed to cause  
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2246 adverse effects on wildlife (Pablos et al. 2015). In contrast, effects on plant populations, especially in  
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2447 environmentally realistic trace amounts, are seldom addressed and do not seem to cause significant  
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2748 inhibition (An et al. 2009). However, low-doses of plant toxins are well-known to have an impact on  
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2949 plant populations. Low-doses of plant toxins can induce stimulatory responses in many plant traits  
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3250 and species (Calabrese and Howe 1976; Duke et al. 2006; Cedergreen et al. 2007; Calabrese and Blain  
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3451 2009). This enhancement in plant performance due to low chemical exposure is believed to be a  
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3652 widespread phenomenon, generally known as hormesis (Calabrese 2008). In order to detect this  
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3953 growth enhancement, one should concentrate on very low concentrations that are below the  
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4154 concentrations causing significant toxic effects.

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4455 Even though such stimulatory responses can be present at the mean population level, the phenomenon  
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4656 does not always seem to occur homogeneously throughout a population in dense plant stands (Belz  
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4957 and Sinkkonen 2016a, b). Moreover, hormesis may remain hidden at the mean population level even  
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5158 though slow-growing individuals with short root/shoot elongation show strong hormetic responses.  
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5359 The associated lack of hormesis among the fast-growing individuals with long root/shoot elongation  
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5660 may be due to a more limited capacity for enhanced growth since these vigorously growing  
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5861 individuals may already have allocated all possible resources to growth (Belz and Sinkkonen 2016a).

62 However, if hormesis is observed at the mean population level, it usually involves a stimulation of  
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263 slow- and fast-growing individuals (Belz and Sinkkonen 2016a).  
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564 Besides hormesis leading to significantly enhanced responses in population mean, another low-dose  
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765 phenomenon may occur for plant toxins leading to significant effects on individual plants within a  
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1066 population without changing the overall response. This phenomenon is called ‘selective low-dose  
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1267 toxicity’ in the case of toxic effects and ‘selective low-dose stimulation’ in the case of stimulatory  
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1468 effects. These selective low-dose effects have been observed to appear differently among individuals  
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1769 having a different growth rate and, thus, whether they are fast- or slow-growing (Sinkkonen et al.  
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1970 2008, 2011). Exposing high-density plant populations to low toxicant concentrations has caused a  
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2271 significant decrease in growth especially in the fast-growing part of a population (Sinkkonen et al.  
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2472 2011; Belz and Sinkkonen 2016a, b). It has been proposed that the higher growth rate of the fast-  
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2773 growing individuals leads to a faster toxicant uptake affecting growth in an adverse manner (Aina et  
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2974 al. 2006).  
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3175 Due to the possibility that environmental pollutants cause such low-dose phenomena in toxin-exposed  
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3476 natural plant populations, there is a risk for long-term environmental consequences. Since low  
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3677 toxicant exposures have been confirmed to inhibit mainly the growth of fast-growing seedlings,  
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3978 Sinkkonen et al. (2011) hypothesized that if natural conditions favor the survival of the fastest  
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4179 growing individuals, a low chemical exposure can drastically affect the overall survival of a plant  
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4480 population because root growth is directly linked to the efficiency of water uptake. The authors  
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4681 confirmed that two ‘emerging contaminants’, namely HHCB [1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-  
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4982 hexamethylcyclopenta(g)-2-benzopyran] and 4-tert-octylphenol [4-(1,1,3,3-  
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5183 tetramethylbutyl)phenol] can cause selective low-dose inhibition on root growth of the most fast-  
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5484 growing individuals of *Gypsophila elegans* Bieb. (annual baby’s breath). As Sinkkonen et al. (2011)  
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5685 did not observe hormesis possibly because of limited replications, and as natural plant populations  
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5886 are commonly exposed to trace amounts of these two toxicants, HHCB and 4-tert-octylphenol were  
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87 chosen to investigate potential selective low-dose effects in more detail and the interplay with a  
1 possible induction of hormesis.

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89 HHCB, also known as galaxolide, is a synthetic musk compound widely used as an ingredient in  
90 consumer products such as cosmetics and fragrances (HERA 2004). It has been detected in both  
91 effluent waters from wastewater treatment plants (Klaschka et al. 2012) and sewage sludge (Kupper  
et al. 2004), which often have applications in agricultural use. Due to its high sorption in soil, low  
leaching and low soil degradation, HHCB is likely to remain in the upper soil layers after being  
applied to soils exposing also a putative risk for terrestrial plants (Litz et al. 2007). In addition, HHCB  
has been observed to affect also root elongation of wheat seedlings and of *Lactuca sativa* L. by  
inhibition at higher and stimulation at lower doses (An et al. 2009; Agathokleous et al. 2018; Belz et  
al. 2018). This biphasic action was said to be caused by the hormone-like characteristics of HHCB  
(An et al. 2009).

4-*tert*-Octylphenol is a high-production volume alkylphenol substance with applications in industrial  
processes, for example in rubber processing or production of ethoxylates which are further used in  
emulsion polymerization or water-based paints (Brooke et al. 2005). The compound is especially  
found in aquatic environments including groundwater (Hernando et al. 2004, Tao et al. 2011), and  
can reach terrestrial environments when soils are irrigated with reclaimed wastewater (Chen et al.  
2013). Furthermore, 4-*tert*-octylphenol seems to accumulate in soils (Chen et al. 2013).  
Ecotoxicological data about the phytotoxicity of 4-*tert*-octylphenol seems to be lacking, yet  
Sinkkonen et al. (2011) observed a significant reduction of root length by low doses of the compound  
among the fast-growing seedlings of a *G. elegans* population. Moreover, 4-*tert*-octylphenol has been  
observed to cause a biphasic response on root elongation of *L. sativa* (Agathokleous et al. 2018; Belz  
et al. 2018). Therefore, when studying low-dose effects of the two emerging contaminants, HHCB  
and 4-*tert*-octylphenol, it is important to manifest possible ecological risks of such pollutant-driven  
low-dose effects on natural vegetation.

112 Previous studies about the selective low-dose effects explored the topic from an agricultural point of  
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123 view. Commercially cultivated plant species or weed species were exposed to herbicides that are  
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124 common in agriculture (Belz and Sinkkonen 2016a, b). Other studies focused on selective low-dose  
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125 toxicity only (Sinkkonen *et al.* 2009, 2011). Therefore, the two main objectives of this study were to  
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126 study the effects of emerging contaminants on a wild plant species, namely the wildflower *G. elegans*,  
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127 and to explore low-dose stimulatory effects in order to better assess possible environmental  
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128 consequences. We focused on the following hypotheses: a) the emerging contaminants HHCB and 4-  
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129 *tert*-octylphenol induce hormesis in a *G. elegans* population; and b) selective low-dose effects and/or  
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120 hormesis appear and vary among the fast- and slow-growing individuals of *G. elegans* even though  
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221 the population mean remains unchanged. Based on previous findings, selective low-dose toxicity  
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242 among the fast-growing seedlings and more pronounced hormesis was expected to occur among the  
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223 slow-growing seedlings, so that these low-dose phenomena would occur heterogeneously within the  
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224 plant population and consequently alter the plant size distribution within the population.  
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## Materials and methods

### Bioassay

An experimental high-density population of *G. elegans* (cv. Covent Garden; Saatgut-Vielfalt, Germany) (total 15,600 seeds; 5.2 seedlings/cm<sup>2</sup>) was used as the test population and exposed in complete dose-response germination experiments to HHCB and 4-*tert*-octylphenol (**Table 1**). Since plants are exposed to HHCB and 4-*tert*-Octylphenol mainly in the upper soil layers, where most plant seeds usually germinate (Litz *et al.* 2007, Chen *et al.* 2013), we chose to expose seeds in a germination bioassay. The test method has been used and published previously (Belz and Sinkkonen 2016a, b). Briefly, the assays were done in 6-well cell culture plates (Cellstar, Greiner bio-one) for 5-d prior to measuring shoot and root elongation as endpoints. One layer of filter paper (MN 615, Macherey-Nagel) was placed inside each well before applying the chemicals. The applied concentrations of the

137 test chemicals were chosen based on preliminary tests and comprised besides an untreated control in  
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138 total 12 concentrations ranging from 0.000067 to 0.67 mM for 4-*tert*-octylphenol (Sigma-Aldrich,  
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139 Germany; purity 97 %) and from 0.0000067 to 2.00 mM for HHCB (Sigma-Aldrich, Germany; purity  
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140 50 %). The number of replicates (one replicate equals one well) per treatment was 24 arranged in  
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141 blocks of six replicates (one 6-well-plate) that were randomly placed in a climate chamber. Due to  
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142 the low water solubility of the test chemicals, the various concentrations were prepared from ethanol  
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143 stock solutions by adding increasing amounts to wells. All plates were left open for 1 d in order to let  
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144 the ethanol evaporate. Then, 60 seeds of *G. elegans* and 1.5 mL of demineralized water were added  
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145 to each well/replicate. With HHCB, 65 seeds/replicate were initially added because of a low  
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146 germination rate in the first experiment with 4-*tert*-octylphenol. For the control treatment, only 1.5  
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147 mL of demineralized water was added. The plates were sealed with parafilm before placing them in  
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148 a completely randomized design into a climate chamber. The climate conditions were set to a  
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149 day/night cycle of 12/12 h starting at 8 am with 24/18 °C and a 12 h light period of 50-70  $\mu\text{mol m}^{-2}$   
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150  $\text{s}^{-1}$  photosynthetic active radiation (PAR). After 2-3 days, the number of seeds was harmonized to 50  
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151 seeds/replicate. After 5 d of exposure, plates were frozen at -20 °C until the shoot and root growth of  
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152 the seedlings was evaluated. The evaluation was done using Fitomed (Castellano et al. 2001). If the  
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153 shoot/root length was <1 mm, it was counted as zero.  
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#### 154 ***Statistical analysis*** 42

155 The statistical analysis applied has been largely used and published previously (for example,  
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156 Sinkkonen *et al.* 2009, 2011; Belz and Sinkkonen 2016a, b), but it was now optimized to consider  
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157 block effects and data normalization. All analyses were done with SAS<sup>®</sup> 9.4. At first, the mean values  
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158 per replicate were calculated for absolute shoot and root length values (mean of the 50 seeds per  
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159 replicate) as well as the percentile (%ile) values per replicate. At the left tail of the size distribution  
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160 (the most short-grown individuals referred to as the slow-growing subpopulations), the 5, 8, 10, 15  
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161 and 20 %iles were calculated for HHCB and the 20, 22, 23 and 25 %iles for 4-*tert*-octylphenol due  
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162 to a high number of ungerminated seeds in this experiment. At the right tail of the size distribution  
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163 (the most long-grown individuals referred to as the fast-growing subpopulations), the 90, 95, 97 and  
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164 99 %iles were calculated for both toxicants. Because at each treatment six replicates were blocked on  
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165 one 6-well-plate, we first analyzed for significant differences between these four blocks within a  
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166 treatment by a univariate analysis of variance (Anova;  $\alpha=0.05$ ). Since absolute and %ile values for  
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167 root and shoot data of both contaminants showed partly significant differences between blocks and  
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168 in parts a non-normal data distribution (*Shapiro-Wilk's test*,  $p>0.05$ ), we decided to consider block  
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169 effects in the further statistical analysis and to transform any non-normal, blocked data via Box-Cox  
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170 power transformation.

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Block effects were considered in the form of calculating a mean value per block based on all six replicates blocked on one plate, so that a treatment was characterized by four block values. The Box-Cox transformation for datasets violating the assumption of normality was done after estimating the optimal value of the transformation parameter  $\lambda$  from the data by the maximum likelihood method ( $-3 < \lambda < 3$ ) using the TRANSREG procedure (Piepho 2009, Osborne 2010, Perla 2016, Damesa et al. 2018). Transformation of data was necessary for seven datasets out of entirely 38. For all datasets transformed, the application of the Box-Cox transformation fixed the problem of violating normality. The mean values per block formed the basis for the evaluations at the subpopulation level. After that, these data were used to calculate the mean response per treatment (mean of the four block values per treatment). This formed the basis for the evaluations at the population level.

### ***Selective low-dose effects***

An ANOVA together with a *Tukey* test ( $\alpha=0.05$ ) was done to exclude the treatments with significantly different absolute mean shoot/root length values per block compared to the control treatment. For those treatments that did not show significant differences in absolute mean values at the population level, %ile values per block were compared by a *Mann-Whitney U* test ( $\alpha=0.05$ ) for significant differences between treatments and the control.



187 **Dose-response modelling**

188 Dose-response relationships were modelled at the population level based on the mean response per  
189 treatment and at the ‘percentile-dependent’ subpopulation level based on percentile values, namely  
190 the mean response per treatment for the 95 %ile and, thus, the fast-growing subpopulation represented  
191 by the most long-grown plants, and for the  $\leq 25$  %ile and, thus, the slow-growing subpopulation  
192 represented by the most short-grown plants. Reduced forms of either a monophasic function (Streibig  
193 1988) (**Eq. 1**) or a hormetic model (Brain and Cousens 1989) was modelled (**Eq. 2**):

194 Eq. (1)  $y = d / (1 + \exp(b * \log(x / ED_{50})))$

195 Eq. (2)  $y = (d + f x) / (1 + \exp(b * \log(x / e)))$

196 where  $d$  corresponds to the mean response of the untreated control,  $f$  reveals the degree of hormetic  
197 increase,  $b$  equals the slope of the decreasing curve part, and  $ED_{50}$  the dose causing 50 % inhibition  
198 while parameter  $e$  does not correspond to any actual biological factor (Brain and Cousens 1989). In  
199 case of a biphasic modelling, the following quantitative features were further deduced using  
200 reparameterizations of Eq. 2 (Schabenberger et al. 1999; Belz and Piepho, 2012, 2013): the  $ED_{50}$ , the  
201 maximum stimulatory response  $y_{\max}$  and the dose  $M$  where stimulation is maximal.

202 Based on the nature of the data, either the mono- or the biphasic dose-response model was used. The  
203 choice of the specific model fitted was primarily based on the significance of parameter  $f$  as indicated  
204 by an estimate of  $f$  with a 95 % confidence interval that did not cover the value zero ( $f > 0$ )  
205 (Schabenberger et al. 1999). If  $f > 0$  was not fulfilled, but the graph of the data for response  $y$  versus  
206 dose  $x$  indicated hormesis, a pairwise likelihood ratio test with the monophasic model (Eq. (1)) as the  
207 reduced model was performed as goodness-of-fit test with the  $p$ -value of the test statistic being  
208 approximated by the chi-square distribution  $\chi^2$  (Seber and Wild 1989; Belz and Piepho 2017).  
209 Additionally, the  $ED_{10}$  dose level was calculated to distinguish the low-dose range from high-dose  
210 inhibitory effects (Streibig and Jensen 2000) (**Eq. 3**):

211 Eq. (3)  $ED_{10} = ED_{50} * (10/100 - 10)^{1/b}$

212 Starting values for the regression parameters were selected based on the graph of the data for response  
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213 y versus dose x. Response variance heterogeneity was accounted for by using the inverse variance of  
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214 replicates at each dose as weight. Significant differences between dose-response curves were  
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215 evaluated by comparing regression parameters using the CONTRAST statement within the  
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216 NLMIXED procedure ( $\alpha=0.05$ ).  
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## 14 **Results**

### 15 ***HHCB***

#### 16 ***Selective low-dose effects***

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22 After an HHCB exposure, seven out of 12 treatments showed no significant difference in terms of  
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25 mean shoot elongation when compared to the control (**Table 2**). Shoot elongation of the slow-growing  
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27 seedlings ( $\leq 20$  %iles) was significantly inhibited at six doses (excluding 0.002 mM) at all percentiles  
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29 tested. The last treatment, however, may already account for beginning of high-dose inhibition. The  
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31 fast-growing seedlings ( $\geq 90$  %iles) were negatively affected only by a dose of 0.013 mM at two  
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33 percentiles tested. This indicates that low-dose toxicity affected shoot elongation by HHCB among  
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35 both slow- and fast-growing seedlings, while selective low-dose stimulation was absent.

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39 Regarding mean root elongation, nine out of 12 treatments were not significantly different from the  
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41 control. Among the slow-growing seedlings, two doses (0.0000067 and 0.00013 mM) negatively  
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43 affected root elongation at two percentiles tested. Root elongation of the fast-growing seedlings was  
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45 selectively inhibited at three doses (0.0000067, 0.013 and 0.067 mM) at all percentiles tested. The  
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47 highest treatment, however, may already account for beginning high-dose inhibition. Hence, results  
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49 indicate that low-dose toxicity by HHCB was less pronounced on root elongation compared to shoot  
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51 elongation.  
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## 235 *Dose-response modelling*

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236 HHCb exposure lead to monophasic responses at the population level for both endpoints measured  
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237 (**Fig. 1; Supplement Table A.1 and A.2**). Regarding shoot growth, the  $ED_{50}$  value was  $0.316\pm 0.031$   
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238  $mM$ . Modelling dose-response curves for the 20 and 95 %ile revealed as well only monophasic  
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239 relationships (**Fig. 2; Supplement Table A.1**). A high variability was observed among the slow-  
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240 growing individuals, so that the biphasic model could not provide a significant fit to the data despite  
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241 a triphasic low-dose trend in the data in the form of a horizontal s-shaped curve in the low-dose range  
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242 with a slight inhibition before the stimulatory peak. However, this kind of dose-response pattern  
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243 cannot be captured well by the current biphasic model (Brain and Cousens 1989), but for example by  
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244 the hormetic model by Cedergreen et al. (2005) which allows the curve to go down before the  
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245 hormetic increase. Nevertheless, due to the high variability the Cedergreen et al. (2005) model could  
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246 not be fitted to the data.

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247 The  $ED_{50}$  for the 20 %ile was  $0.076\pm 0.026 mM$  and, thus, significantly lower (4.2-fold) as compared  
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248 to the  $ED_{50}$  of the entire population indicating a higher sensitivity of the slow-growing seedlings. The  
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249 fast-growing part seemed to be less prone to HHCb and showed an  $ED_{50}$  of  $0.876\pm 0.074 mM$   
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250 corresponding to a 2.8-fold higher value compared to the mean population and 11.5-fold compared  
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251 to the slow-growing seedlings. Hence, the more vigorously growing seedlings were significantly less  
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252 sensitive to HHCb than most of the population and needed considerably higher doses to be inhibited.

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253 Therefore, shoot elongation of the slow- and fast-growing seedlings clearly showed selective dose  
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254 inhibition after an HHCb exposure and indicated an impact on the size distribution.

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255 Regarding root growth, the  $ED_{50}$  value at the population level was  $0.216\pm 0.025 mM$ . Modelling dose-  
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256 response curves for the 10 and 95 %ile revealed only monophasic relationships that did however not  
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257 significantly differ in  $ED_{50}$  from the population mean (**Fig. 2**). A high variability occurred again  
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258 among the slow-growing individuals so that any visible bi- or triphasic trend in the data could not be  
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259 significantly modelled. The  $ED_{50}$  for the 10 %ile was  $0.267\pm 0.195 mM$ . Root growth of the fast-  
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260 growing individuals was more homogenous and showed an  $ED_{50}$  of  $0.200\pm 0.013$  mM. For that reason,  
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261 HHCb exposure did not cause selective effects on root elongation of the slow- and fast-growing  
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262 seedlings.  
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### 263 **4-tert-Octylphenol**

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#### 264 **Selective low-dose effects**

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11 According to *Tukey* test, the mean shoot growth of four treatments was not statistically different from  
12  
13 the control (**Table 3**). Regarding the slow-growing seedlings ( $\leq 25$  %iles), no significant selective  
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15 low-dose effects occurred. Shoot growth of the most fast-growing seedlings ( $\geq 90$  %iles) was also not  
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17 selectively stimulated, but inhibited at two doses (0.002 and 0.02 mM) at all percentiles tested. This  
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20 indicated the presence of some low-dose toxicity on shoot growth in the fast-growing subpopulations,  
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23 although not very prevalent.  
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26 Regarding mean root elongation, seven out of 12 treatments were not significantly different from the  
27  
28 control (**Table 3**). Root growth of the slow-growing seedlings showed no significant selective low-  
29  
30 dose effects. Among the fast-growing seedlings, five treatments caused significant inhibition at  
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32 between one and all percentiles tested (0.000067, 0.00067, 0.002, 0.0067 and 0.02 mM). This  
33  
34 widespread selective inhibition of root elongation among mainly the fast-growing part of the  
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37 population clearly indicated the presence of low-dose toxicity, while selective low-dose stimulation  
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40 was again absent.  
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#### 43 **Dose-response modelling**

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46 4-tert-Octylphenol exposure led to monophasic responses at the population level for both endpoints  
47  
48 measured (**Fig. 3; Supplement Table A.1 and A.2**). The  $ED_{50}$  value for shoot elongation was  
49  
50  $0.099\pm 0.008$  mM. Modelling dose-response curves for shoot growth at the 25 and 95 %ile revealed  
51  
52 as well only monophasic relationships (**Fig. 4; Supplement Table A.1**). A high variability was again  
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54 observed among the slow-growing 25 %ile showing an  $ED_{50}$  of  $0.062\pm 0.018$  mM. This value was  
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57 significantly lower (1.6-fold) as compared to the  $ED_{50}$  of the entire population indicating a higher  
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285 sensitivity of the slow-growing seedlings. The response of the fast-growing part (95 %ile) seemed to  
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286 be more stable with an  $ED_{50}$  for shoot elongation of  $0.117 \pm 0.008$  mM. This value was not significantly  
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287 higher than that of the mean population, but corresponded to a 1.9-fold higher value compared to the  
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288 slow-growing seedlings. Therefore, the most fast-growing seedlings were less sensitive to 4-*tert*-  
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289 octylphenol than the slow-growing seedlings and needed considerably higher doses in order to be  
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Regarding root growth, the  $ED_{50}$  value for 4-*tert*-octylphenol at the population level was  $0.102 \pm 0.008$  mM (**Fig. 3**). As to the subpopulation levels (23 and 95 %ile), modelling dose-response curves for root length revealed that a biphasic dose-response curve provided the best fit for the slow-growing seedlings despite an insignificant  $f$  value, while responses of the fast-growing seedlings were monophasic (**Fig. 4; Supplement Table A.2**). The maximum stimulation ( $y_{max}$ ) was  $185 \pm 51$  % at a dose  $M$  of  $5.8 \pm 2.7$   $\mu$ M. The  $ED_{50}$  value for the slow-growing seedlings was  $0.074 \pm 0.037$  mM, which was not significantly different compared to the  $ED_{50}$  of the mean population. Considering the fast-growing 95 %ile, the  $ED_{50}$  for root growth was  $0.124 \pm 0.008$  mM. This value was significantly higher as compared to the mean population (1.2-fold) and the slow-growing subpopulation (1.7-fold). Consequently, there was some selective hormesis in the population albeit restricted to the slow-growing seedlings and masked at the population level. The most fast-growing seedlings were also significantly less sensitive to 4-*tert*-octylphenol in root growth as most of the population.

## Discussion

This study aimed at investigating selective low-dose toxin effects and dose-dependent selectivity from an environmental perspective by exposing a wild plant population to two emerging contaminants at environmentally relevant concentrations. The two chosen toxicants did not cause selective low-dose stimulation without changing the mean plant size and did not induce significant hormesis in root or shoot growth despite one exception observed with 4-*tert*-octylphenol at the slow-growing

310 subpopulation. A wide absence of hormesis does not necessarily mean that a compound is generally  
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311 not hormetic since there are several factors that influence the occurrence and expression of a hormetic  
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312 response in plants (Belz and Piepho 2014). Changing the experimental setup in terms of a prolonged  
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313 timeframe, different test parameters, or several lower concentrations can sometimes reveal hormesis.  
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314 Microbial interactions, resource competition and pests can be the reason for the lack of hormesis in  
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315 many plant stands (Hansi et al. 2014, Płociniczak et al. 2013, 2016, Yu et al. 2015). Additionally, the  
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316 expression of stimulatory responses seems to be linked to the species or biotype used, so that some  
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317 plants are simply prone to lack hormesis. For example, Rodriguez et al. (2012) identified one  
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318 quantitative trait locus (QTL) on a chromosome that caused the natural variation and the lack of  
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319 hormesis in the heat-stress response of different biotypes of the nematode *Caenorhabditis elegans*  
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320 Maupas. This finding suggests that natural variation in hormesis and its absence may have a genetic  
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321 background. Furthermore, the lack of hormesis can also be due to growth conditions since both poor  
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322 and exceptionally optimized conditions have been shown to cause the absence of hormesis even  
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323 though a compound would otherwise induce hormesis (Belz and Cedergreen 2010). For instance, an  
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324 increase in leaf area of *Sinapis arvensis* L. (wild mustard) under a parthenin exposure was lacking  
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325 during a non-optimal warm period, yet the enhancement occurred under cooler conditions (Belz  
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326 2008). The same phenomenon was observed with glyphosate-exposed *Hordeum vulgare* L. (barley)  
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327 by altering the CO<sub>2</sub> supply. The amount of biomass of *H. vulgare* in response to glyphosate did not  
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328 increase at below ambient concentrations even though the hormetic response was observed at ambient  
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329 and even higher CO<sub>2</sub> levels (Cedergreen and Olesen 2010). Additionally, a study conducted with  
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330 parthenin-exposed *Lactuca sativa* L. (lettuce) revealed that hormesis was absent under exceptionally  
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331 good growing conditions (Belz and Cedergreen 2010). It has been surmised that the cell growth rate  
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332 under optimal conditions is already at maximum and cannot be further enhanced (Vichi and Tritton  
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333 1989). Hence, a toxicant-induced hormesis seems to be most pronounced at below maximal, but still  
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334 at favorable environmental conditions.  
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335 Selective low-dose toxicity of mainly fast-growing seedlings seemed to be characteristic for low-dose  
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336 effects of HHCb and 4-*tert*-octylphenol on *G. elegans*, although this phenomenon was not very  
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337 prevalent. Previous studies by Sinkkonen et al. (2008, 2011) with the same contaminants were ten  
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338 times smaller in scale than the current well replicated study and used separate individuals as  
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339 replicates, while the current study uses percentile and mean values per dish as replicates. Furthermore,  
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340 previous studies used commercially cultivated plant species or weed species rather than a more  
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341 heterogeneous, wild plant population. Therefore, this study clearly indicates that the phenomenon of  
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342 selective low-dose effects within a population also holds true under ecologically more relevant  
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343 conditions and represents a further step towards the elucidation of the ecological significance of this  
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344 low-dose phenomenon.  
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345 Low-dose toxicity may be linked to density-dependent phytotoxicity, so that when plants share the  
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346 same toxicant pool in dense plant stands (Weidenhamer et al. 1989, Sinkkonen 2001, 2003), the fast-  
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347 growing individuals can take up higher amounts of toxicants due to their higher activity (Sinkkonen  
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348 et al. 2009). Regarding high-dose selective toxicity, the slow-growing seedlings showed the highest  
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349 sensitivity to the chosen compounds, followed by the mean population, while the fast-growing  
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350 subpopulation seemed to be the most inert part of the population. This pattern of sensitivity was rather  
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351 consistent throughout our findings irrespective of the compound or endpoint investigated. A decrease  
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352 in sensitivity among the fast-growing subpopulation and a respective increased sensitivity among the  
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353 slow-growing seedlings has also been observed in previous studies using other test species and  
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354 chemicals (Belz and Sinkkonen 2016a). Nonetheless, compared to our previous studies with other  
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355 toxins and plant species, both compounds showed a rather low capacity to differentiate populations  
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356 of *G. elegans*, especially at low doses.  
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### 357 ***Selective effects of HHCb exposure*** 54

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358 Under an HHCb exposure, the shoot growth of the slow-growing subpopulation was adversely  
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359 affected by several different doses, yet the fast-growing subpopulation remained widely unaffected  
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360 whether shoot or root growth was considered. Based on this, it seems that there is some low-dose  
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361 toxicity with HHCb, but it did not seem to be as prevalent and characteristic mainly for the fast-  
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362 growing subpopulation as expected from earlier findings with other plant species. This indicated that  
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363 the pattern and expression of selective low-dose effects may depend on the effective compound and/or  
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364 the exposed plant species. The finding that *G. elegans* is rather inert to low-dose selective effects of  
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365 HHCb corresponds to previous reports where low-dose toxicity of HHCb was also not very  
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366 pronounced (Sinkkonen et al. 2011).  
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367 The detected environmental concentration of 0.00631  $\mu\text{M}$  (**Table 1**) (Litz et al. 2007) closely  
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368 corresponds to the lowest dose actually tested and causing significant, selective inhibition of shoot  
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369 growth in both fast- and slow-growing seedlings (0.0067  $\mu\text{M}$ ). Although the observed  $ED_{50}$  values  
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370 are several magnitudes higher, this observation clearly substantiates the environmental significance  
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371 of low-dose exposures of HHCb in terms of alterations in size distribution of exposed plant  
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372 populations by selective low-dose toxicity.  
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### 31 ***Selective effects of 4-tert-octylphenol exposure*** 32

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374 Regarding 4-tert-octylphenol, selective low-dose toxicity occurred more profoundly among the fast-  
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375 growing subpopulation since both shoot and root elongation were selectively inhibited at certain low-  
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376 doses, while the slow-growing part of the population remained unaffected. Especially root growth of  
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377 the fast-growing seedlings showed selective low-dose toxicity by 4-tert-octylphenol, which is in line  
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378 with a previous study showing significant reduction in root length of *G. elegans* among the fast-  
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379 growing subpopulation after 4-tert-octylphenol exposure (Sinkkonen et al. 2011). This is an important  
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380 revelation as the previous study was produced in a different laboratory using another root  
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381 measurement method. Therefore, the current study finds the first proof that selective low-dose toxicity  
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382 is a persistent phenomenon.  
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383 Regarding selective hormetic effects, 4-tert-octylphenol was only stimulatory towards root growth of  
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384 slow-growing *G. elegans* plants with a maximum of 85 % stimulation above control. Because this  
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385 selective enhancement of growth was masked at the population level, the results confirm the  
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386 assumption that the stimulation of fast-growing seedlings is the decisive factor for the formation of  
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387 hormesis at the population level (Belz and Sinkkonen 2016a, b). Additionally, the  $ED_{50}$  values  
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388 revealed significant differences in sensitivity of the different subpopulations compared to the mean  
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389 population with the slow-growing seedlings being the most sensitive group and then the fast-growing  
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so that 4-*tert*-octylphenol would not seem to cause either adverse or stimulatory selective effects on *G. elegans* at such environmental levels.

### ***Dose-response modelling***

For several curves generated for HHCB, there was clearly a trend that a slight decrease in response was followed by an enhancement in both shoot and root lengths not only among the slow-growing seedlings, but also fast-growing ones and even at the mean population level. This so called triphasic dose-response cannot be captured by a monophasic model (Streibig 1988), which widely ignores this low-dose depression in responses. However, to a certain extent the biphasic model of Cedergreen et al. (2005) is flexible enough to capture a triphasic pattern (Belz and Piepho 2012). In this study, a triphasic pattern could however not be significantly fitted whether the dose-response modelling was based on the individual values from each replicate per treatment or the mean values per treatment in order to dampen the variability observed in the data especially at the low percentiles. This apparent lack of a better fit than the monophasic model may account for the partly high variability of responses, but also an insufficient number of treatments covering the observed low-dose depression. In the latter case, a triphasic curve is not easy to model which is probably why triphasic curve shapes are seldom

409 reported. However, there are some previous studies showing this phenomenon (Belz and Piepho  
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410 2012).

### 411 ***Practical implications***

412 Previous studies performed with commercially cultivated *L. sativa* have established the parallel  
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413 occurrence of hormesis, selective low-dose toxicity and selective low-dose stimulation within a dense  
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414 plant population (Belz and Sinkkonen 2016a). Based on the genetic homogeneity of a cultured species  
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415 such as *L. sativa*, these findings should be applied carefully to natural plant populations since the  
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416 genetic variation of wild plants is expected to be more pronounced (Belz and Sinkkonen 2016a).  
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417 Nevertheless, the hypothesis that low toxicant levels can segregate certain plant populations has  
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418 already been confirmed with an agricultural weed population (Belz and Sinkkonen 2016b). Compared  
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419 to this, our results showed rather weak low-dose effects of the chosen compounds on populations of  
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420 *G. elegans*. This provokes the question of whether this is due to the compounds tested or the plant  
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421 species used. Hormetic effects on plants have not been studied before using HHCB and 4-tert-  
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422 octylphenol, so that it is unknown if the observed lack of hormesis is a common situation. However,  
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423 *G. elegans*, as a wild plant, expressed a rather pronounced variation response-wise, which made it  
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424 difficult to observe significant low-dose effects. The high variability of responses among the slow-  
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425 growing seedlings seemed to disturb the detection of significant low-dose effects and thus acted as  
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426 an argument for focusing on higher %iles for dose-response modelling compared to our previous  
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427 studies. Additionally, due to the relative slow development of *G. elegans* compared to the previously  
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428 studied fast-developing *L. sativa* and the more pronounced occurrence of selective low-dose toxicity  
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429 in *L. sativa* (Belz and Sinkkonen 2016a), it can be hypothesized that species with a low overall growth  
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5130 rate are less prone to develop selective low-dose effects.

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5331 Since there is a vast amount of harmful chemicals at low doses in the environment (for example,  
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5632 Klaschka et al. 2012), the likelihood that natural vegetation is exposed to compounds causing  
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5833 selective effects at low-doses seems to be very realistic. The importance of studying the topic of low-  
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434 dose effects has been emphasized before (Belz and Sinkkonen 2016a), especially in relation to  
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435 extreme weather conditions such as drought, since a change in root size distribution is of utmost  
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436 importance for the efficiency of water uptake and, thus, the survival of plants (Sinkkonen et al. 2009).  
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437 If a population is simultaneously exposed to both drought and low toxicant doses, the fast-growing  
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438 seedlings seem to be more prone to an inhibition of root growth, as observed in this study. This can  
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439 decrease the overall survival of that plant population, such that the plants that would be the most  
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440 likely to survive from drought are now inhibited and may not be able to contribute to the survival of  
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441 the whole plant population. Additionally, in natural conditions, chemical exposure is likely to be  
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442 rather continuous, especially since both of the studied compounds have shown a tendency to persist  
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443 in soil (Litz et al. 2007, Chen et al. 2013). This kind of prolonged low-dose exposure could cause  
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444 further effects even on more slow-growing species such as *G. elegans*. Overall, it has been surmised  
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445 that such low-dose driven changes in the structure of a population are directly related to plant  
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446 performance and survival under extreme environmental conditions (Chu et al., 2008, 2009) and,  
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447 hence, ultimately to reproduction. This may change population dynamics and lead to genotypic  
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448 adaptations and/or ecotype formation in the longer term with ecologically significant consequences  
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449 for the ecosystem or biodiversity (Sinkkonen et al. 2009).  
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450 Despite the fact that toxicant levels detected from the environment tend to be rather low, usually in  
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451 the ng/L to µg/L range (Klaschka et al. 2012, Tao et al. 2011), it indeed seems that such negligible  
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452 concentrations have the potential to lead to negative growth effects within a plant population even  
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453 though such concentrations would have been considered safe in ecotoxicological bioassays. A similar  
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454 trend was observed by Sinkkonen et al. (2009) who further suggested that current laboratory standards  
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455 in risk evaluation should include the possibility of low-dose effects and that re-evaluation of the  
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456 threshold levels for environmental contaminants would be needed. One of the lowest predicted no  
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457 effect concentrations (PNEC) in aquatic environments regarding HHCB is 0.23 µM for the fish  
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458 *Pimephales promelas* Rafinesque (fathead minnow) (HERA 2004). As we observed adverse selective  
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459 effects at levels that were tens of times lower, the current study implicates a need to re-evaluate the  
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460 test protocol of PNECs.

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## 462 **Supplementary data**

463 **Table A.1** Regression parameters from the monophasic modeling (Streibig 1988) of toxin effects on  
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12 shoot growth of *Gypsophila elegans*. Data given as mean  $\pm$  standard error.

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465 **Table A.2** Regression parameters and estimated quantitative features from the monophasic (Streibig  
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18 1988) or biphasic (Brain and Cousens 1989) modeling of toxin effects on root growth of *Gypsophila*  
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467 *elegans*. Data given as mean  $\pm$  standard error.

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478 40333/14).

479 Conflict of Interest: All authors declare that they have no conflict of interest.

480 Ethical approval: This article does not contain any studies with human participants or animals  
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481 performed by any of the authors.

482

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627 **Tables**1  
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3629 **Table 1** Some chemical properties and environmental concentrations of HHCB and 4-*tert*-  
630 octylphenol.630  
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| Chemical name  | Formula                           | Molecular weight [g/mol] | CAS no.   | Effluent [ $\mu$ M]  | Sludge [mg/kg d.m.]    |
|--|-----------------------------------|--------------------------|-----------|----------------------|------------------------|
| <b>HHCB</b>  |                                   |                          |           |                      |                        |
| 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran | C <sub>18</sub> H <sub>26</sub> O | 258.41                   | 1222-05-5 | 0.00631 <sup>1</sup> | 20.3 <sup>2</sup>      |
| <b>4-<i>tert</i>-octylphenol</b>                                       |                                   |                          |           |                      |                        |
| 4-(1,1,3,3-tetramethylbutyl)phenol                                     | C <sub>14</sub> H <sub>22</sub> O | 206.32                   | 140-66-9  | 0.00044 <sup>3</sup> | 0.08-0.20 <sup>4</sup> |

631 <sup>1</sup> Klascka *et al.* 2012, <sup>2</sup> Kupper *et al.* 2004, <sup>3</sup> Höhne *et al.* 2008, <sup>4</sup> Bolz *et al.* 2001; d.m.=dry mass631  
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633 **Table 2** Statistical significance of low-dose effects of HHCB on shoot and root length of *Gypsophila*  
634 *elegans* at different percentiles. Displayed are only concentrations for which mean root/shoot length  
635 at the population level was not significantly different from control (*Tukey*-test at  $\alpha=0.05$  for four  
636 blocks with six replicates per concentration (mm)).636  
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| End-point         | Dose [mM] | Mean | Percentile |      |      |      |       |       |       |       |       |
|-------------------|-----------|------|------------|------|------|------|-------|-------|-------|-------|-------|
|                   |           |      | 5 %        | 8 %  | 10 % | 15 % | 20 %  | 90 %  | 95 %  | 97 %  | 99 %  |
| shoot length [mm] | control   | 6.6  | 0.8        | 1.5  | 2.0  | 3.0  | 3.8   | 10.6  | 11.6  | 12.0  | 12.9  |
|                   | 0.0000067 | 5.8  | 0.1*       | 0.3* | 0.6* | 2.0* | 3.1   | 9.7   | 10.6  | 11.2  | 12.1  |
|                   | 0.000067  | 6.2  | 0.3        | 0.5  | 1.1* | 2.3  | 2.9   | 10.5  | 11.7  | 12.2  | 13.1  |
|                   | 0.00067   | 6.3  | 0.2        | 0.6* | 1.1* | 2.1  | 3.1   | 10.5  | 11.4  | 11.9  | 12.6  |
|                   | 0.002     | 6.8  | 0.7        | 1.3  | 1.8  | 3.0  | 4.0   | 11.0  | 11.7  | 12.4  | 13.3  |
|                   | 0.0067    | 6.1  | 0.0*       | 0.2* | 0.3* | 1.1* | 2.0*  | 10.8  | 11.9  | 12.5  | 13.6  |
|                   | 0.013     | 5.4  | 0.3        | 0.5  | 0.8* | 1.4* | 2.3   | 9.3*  | 10.3* | 10.8  | 11.7  |
|                   | 0.02      | 5.6  | 0.3        | 0.7* | 0.9* | 1.5* | 2.3   | 9.8   | 10.7  | 11.2  | 12.1  |
| root length [mm]  | control   | 13.3 | 0.8        | 1.5  | 1.8  | 3.6  | 5.4   | 23.5  | 25.6  | 27.2  | 30.4  |
|                   | 0.0000067 | 11.5 | 0.1*       | 0.4  | 1.3  | 2.7* | 4.2   | 20.7* | 23.5* | 25.0* | 27.7* |
|                   | 0.000067  | 12.2 | 0.8        | 1.4  | 1.6  | 3.0  | 4.4   | 22.8  | 25.6  | 27.2  | 29.6  |
|                   | 0.00013   | 11.7 | 0.3        | 0.7  | 1.2  | 2.5* | 4.1   | 22.1  | 24.7  | 26.4  | 28.9  |
|                   | 0.00067   | 12.8 | 0.5        | 1.1  | 2.0  | 3.7  | 5.4   | 22.9  | 25.4  | 26.4* | 28.7  |
|                   | 0.002     | 14.0 | 1.1        | 2.0  | 2.8  | 4.8  | 6.4   | 23.9  | 26.3  | 28.2* | 30.9  |
|                   | 0.0067    | 13.1 | 0.2        | 0.9  | 1.4  | 2.9  | 4.8   | 23.9  | 27.0  | 28.2  | 30.4  |
|                   | 0.013     | 12.0 | 0.5        | 1.0  | 1.9  | 3.3  | 4.6   | 21.6  | 23.8  | 25.1  | 27.0* |
| 0.02              | 12.5      | 0.9  | 1.7        | 2.3  | 3.4  | 4.7  | 22.5  | 25.1  | 26.4  | 29.1  |       |
| 0.067             | 11.3      | 0.9  | 1.6        | 1.9  | 3.2  | 4.4  | 20.8* | 22.8* | 24.0* | 25.9* |       |

637 \*significantly different from control according to *Mann-Whitney U* test at  $\alpha=0.05$ .637  
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638 **Table 3** Statistical significance of low-dose effects of 4-*tert*-octylphenol on shoot and root length of  
 639 *Gypsophila elegans* at different percentiles. Displayed are only concentrations for which mean  
 640 root/shoot length at the population level was not significantly different (ns) from control (*Tukey*-test  
 641 at  $\alpha=0.05$  for four blocks with six replicates per concentration (mm)).

| End-point            | Dose           | Mean        | Percentile |            |            |            |             |             |             |             |
|----------------------|----------------|-------------|------------|------------|------------|------------|-------------|-------------|-------------|-------------|
|                      | [mM]           |             | 20 %       | 22 %       | 23 %       | 25 %       | 90 %        | 95 %        | 97 %        | 99 %        |
| shoot length<br>[mm] | <b>control</b> | <b>7.0</b>  | <b>1.4</b> | <b>1.6</b> | <b>1.9</b> | <b>2.6</b> | <b>12.2</b> | <b>13.0</b> | <b>13.5</b> | <b>14.8</b> |
|                      | 0.000067       | 6.3         | 0.2        | 0.5        | 0.9        | 2.0        | 11.6        | 12.6        | 13.3        | 14.5        |
|                      | 0.00067        | 6.8         | 1.3        | 2.0        | 2.2        | 2.4        | 12.4        | 13.7        | 14.6        | 15.7        |
|                      | 0.002          | 6.2         | 1.5        | 2.0        | 2.2        | 2.4        | 10.9*       | 11.7        | 12.3        | 13.3        |
|                      | 0.02           | 5.9         | 1.5        | 1.7        | 1.9        | 2.5        | 10.6*       | 11.5*       | 12.1*       | 13.1*       |
| root length<br>[mm]  | <b>control</b> | <b>13.3</b> | <b>1.4</b> | <b>1.7</b> | <b>2.0</b> | <b>3.0</b> | <b>27.6</b> | <b>30.7</b> | <b>32.4</b> | <b>34.7</b> |
|                      | 0.000067       | 12.0        | 0.3        | 0.6        | 1.0        | 2.3        | 24.7*       | 28.1*       | 29.3*       | 31.0*       |
|                      | 0.00067        | 12.5        | 1.3        | 2.0        | 2.2        | 2.7        | 26.1*       | 28.8*       | 30.6        | 33.7        |
|                      | 0.002          | 13.1        | 1.7        | 2.4        | 2.7        | 3.3        | 26.0*       | 28.9*       | 31.1        | 34.8        |
|                      | 0.0033         | 12.7        | 0.8        | 1.5        | 1.9        | 2.4        | 26.9        | 29.7        | 32.0        | 35.0        |
|                      | 0.0067         | 12.2        | 1.5        | 2.2        | 2.5        | 3.0        | 25.3*       | 28.5*       | 30.3        | 33.0        |
|                      | 0.013          | 12.0        | 1.0        | 1.2        | 1.4        | 1.8        | 26.4        | 29.6        | 31.3        | 34.2        |
|                      | 0.02           | 12.3        | 1.5        | 1.9        | 2.3        | 3.3        | 25.7*       | 30.2        | 32.2        | 35.5        |

642 **\***significantly different from control according to *Mann-Whitney U* test at  $\alpha=0.05$ .

644 **Figure Captions**

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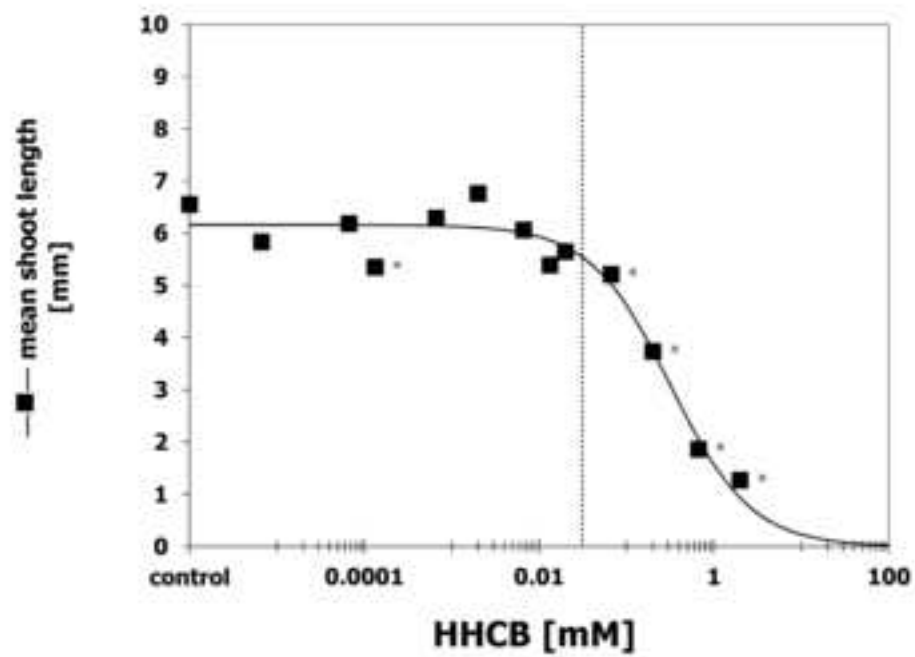
**Fig. 1** Dose-response curves for the effects of HHCb on shoot length (a) and on root length (b) of *Gypsophila elegans* at the population level. The dotted line shows the  $ED_{10}$  value which distinguishes the low-dose range from high-dose inhibitory effects. Doses that are significantly different from the control are marked with an asterisk ‘\*’ (*Tukey* test,  $\alpha = 0.05$ ).

**Fig. 2** Dose-response curves for the effects of HHCb on shoot length of *Gypsophila elegans* at the 20 and 95 % percentiles (%iles) (a) and on root length at the 10 and 95%ile (b) of the tested population. The dotted line shows the  $ED_{10}$  value which distinguishes the low-dose range from high-dose inhibitory effects. Doses that are significantly different from the control are marked with an asterisk ‘\*’ (*Mann-Whitney U* test,  $\alpha = 0.05$ ).

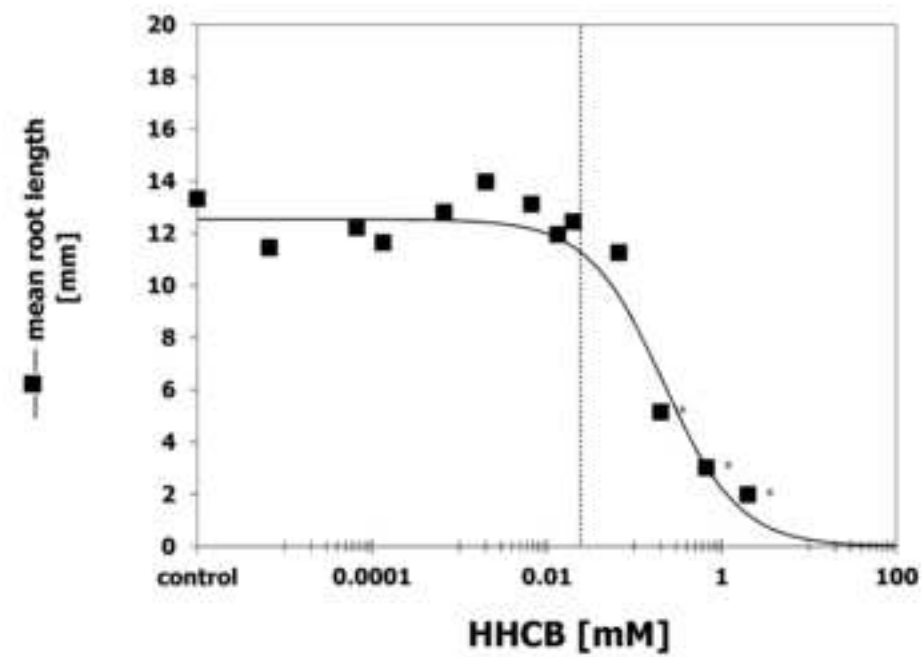
**Fig. 3** Dose-response curves for the effects of 4-*tert*-octylphenol on shoot length (a) and on root length (b) of *Gypsophila elegans* at the mean population level. The dotted line shows the  $ED_{10}$  value which distinguishes the low-dose range from high-dose inhibitory effects. Doses that are significantly different from the control are marked with an asterisk ‘\*’ (*Tukey* test,  $\alpha = 0.05$ ).

**Fig. 4** Dose-response curves for the effects of 4-*tert*-octylphenol on shoot length of *Gypsophila elegans* at the 25 and 95 %iles (a) and on root length at the 23 and 95%ile (b) of the tested population. The dotted line shows the  $ED_{10}$  value which distinguishes the low-dose range from high-dose inhibitory effects. Doses that are significantly different from the control are marked with an asterisk ‘\*’ (*Mann-Whitney U* test,  $\alpha = 0.05$ ).

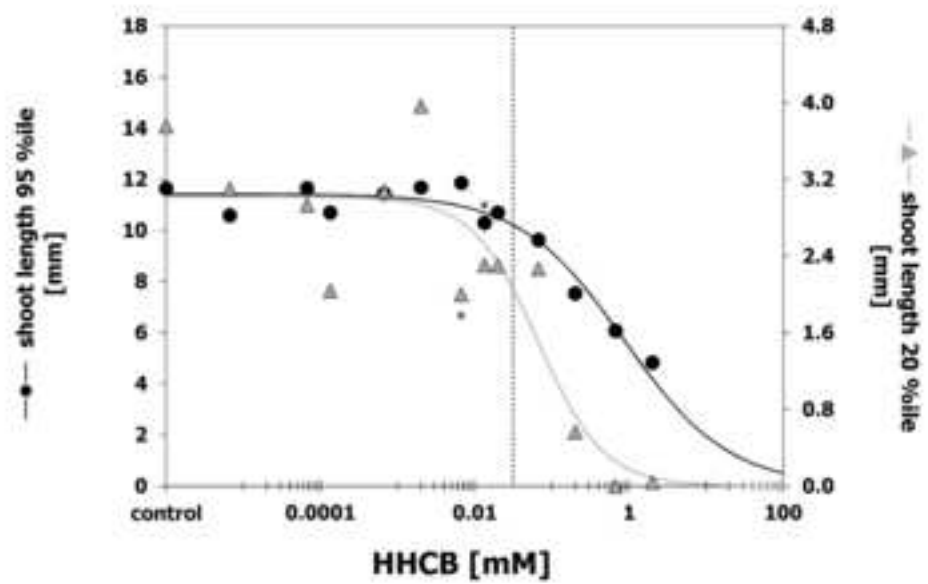
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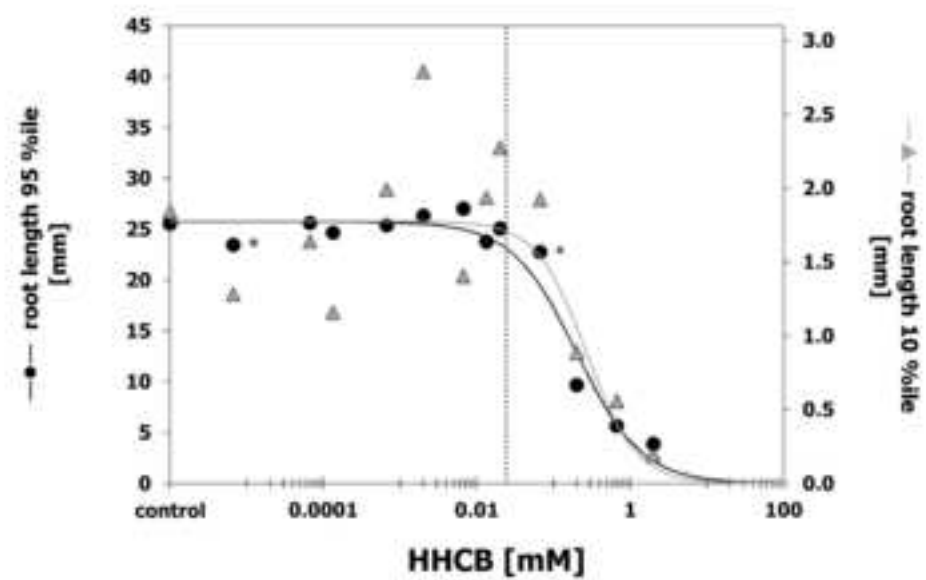
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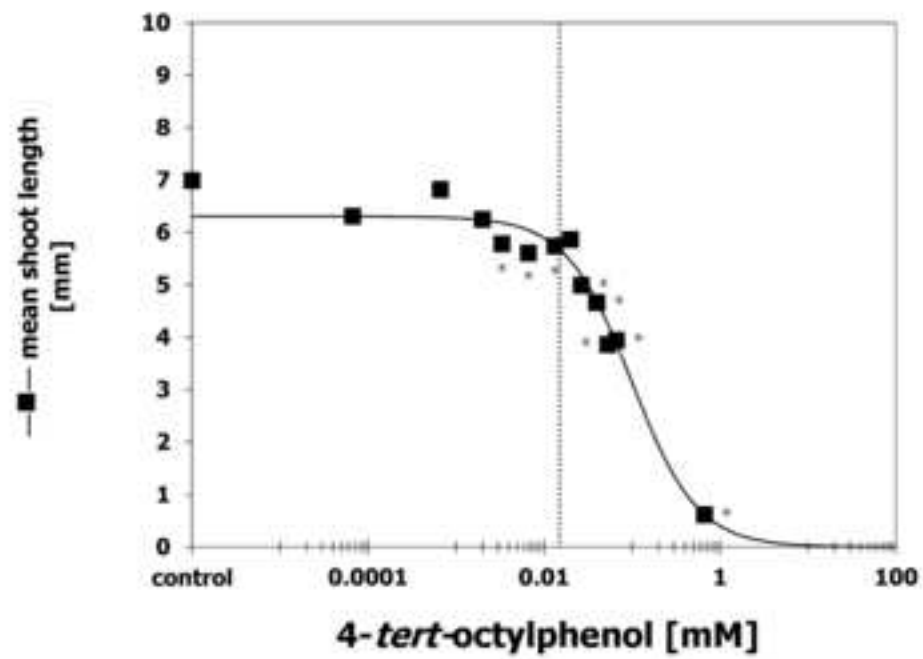
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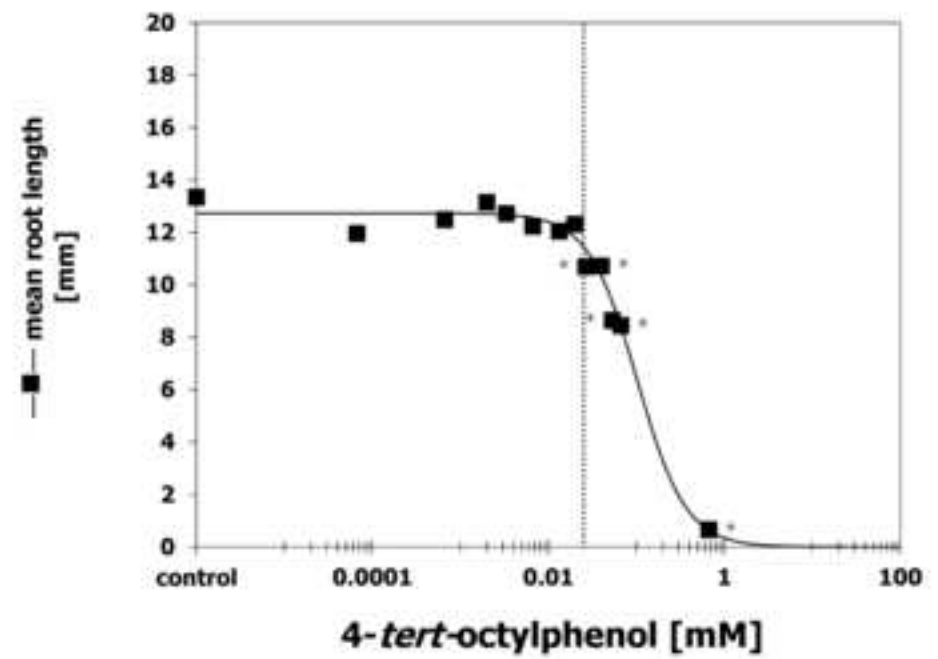
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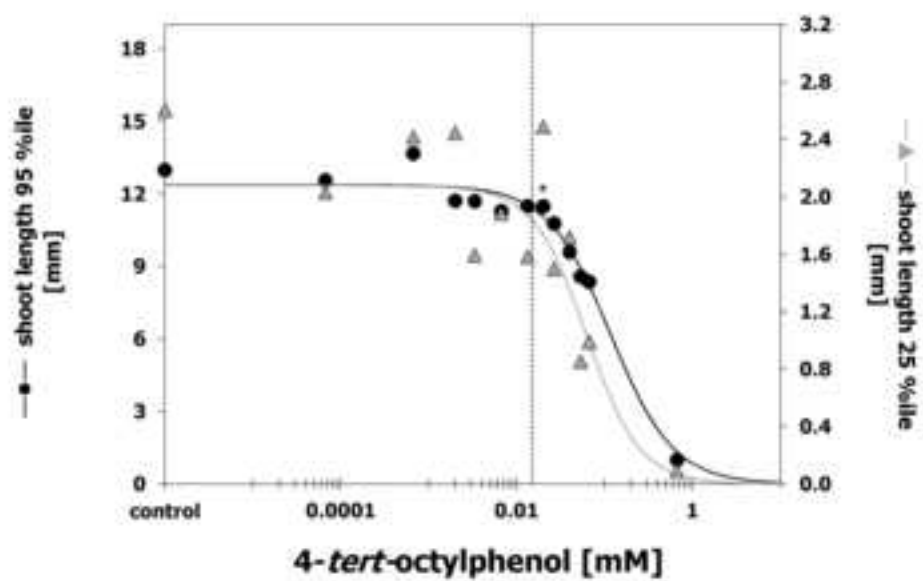


b

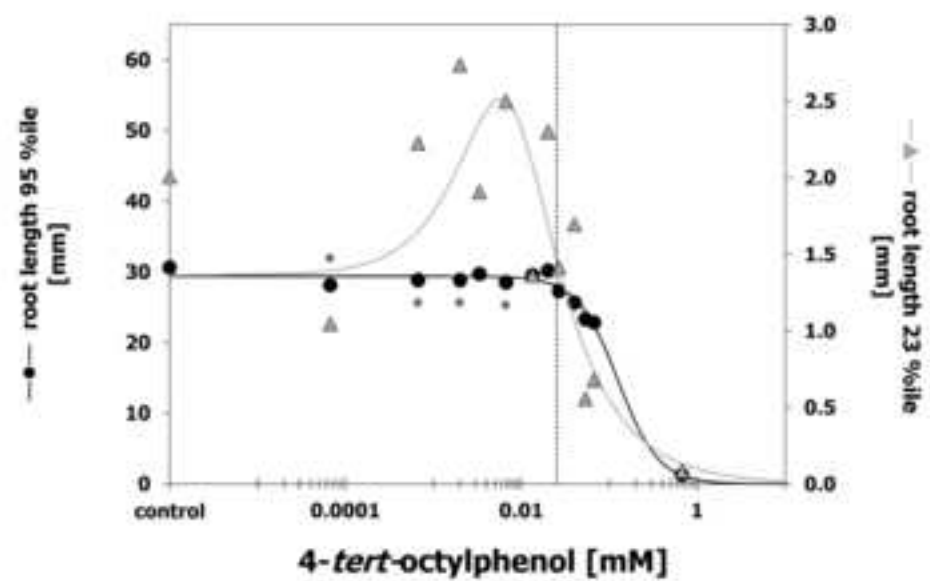




a



b



**Supplement Table A.1** Regression parameters from the monophasic modeling (Streibig 1988) of toxin effects on shoot growth of *Gypsophila elegans*. Data given as mean  $\pm$  standard error.

| toxin              | percentile      | model providing best fit | $d$ [mm]         | $b$             | $ED_{50}$ [mM]      | $ED_{50}$ 95% CI [mM] |
|--------------------|-----------------|--------------------------|------------------|-----------------|---------------------|-----------------------|
| HHCB               | 20%             | monophasic               | 3.01 $\pm$ 0.16  | 1.17 $\pm$ 0.32 | 0.076 $\pm$ 0.026 c | 0.024-0.127           |
|                    | 95%             | monophasic               | 11.46 $\pm$ 0.10 | 0.60 $\pm$ 0.03 | 0.876 $\pm$ 0.074 b | 0.732-1.021           |
|                    | population mean | monophasic               | 6.17 $\pm$ 0.09  | 0.91 $\pm$ 0.08 | 0.316 $\pm$ 0.031 a | 0.256-0.376           |
| 4-tert-octylphenol | 25%             | monophasic               | 2.08 $\pm$ 0.19  | 1.43 $\pm$ 0.68 | 0.062 $\pm$ 0.018 b | 0.026-0.098           |
|                    | 95%             | monophasic               | 12.37 $\pm$ 0.15 | 1.22 $\pm$ 0.11 | 0.117 $\pm$ 0.008 a | 0.101-0.133           |
|                    | population mean | monophasic               | 6.31 $\pm$ 0.11  | 1.16 $\pm$ 0.07 | 0.099 $\pm$ 0.008 a | 0.084-0.115           |

CI = confidence interval; small letters indicate significant differences between  $ED_{50}$  values at  $\alpha=0.05$

**Supplement Table A.2** Regression parameters and estimated quantitative features from the monophasic (Streibig 1988) or biphasic (Brain and Cousens 1989) modeling of toxin effects on root growth of *Gypsophila elegans*. Data given as mean  $\pm$  standard error.

| toxin              | percentile      | model providing best fit | $d$ [mm]         | $b$             | $f$           | $M$ [ $\mu$ M]    | $ED_{50}$ [mM]      | $ED_{50}$ 95% CI [mM] | $y_{max}$ [%] |
|--------------------|-----------------|--------------------------|------------------|-----------------|---------------|-------------------|---------------------|-----------------------|---------------|
| HHCB               | 10%             | monophasic               | 1.78 $\pm$ 0.24  | 1.34 $\pm$ 1.00 | n.s.          | -                 | 0.267 $\pm$ 0.195 a | -0.118-0.651          | -             |
|                    | 95%             | monophasic               | 25.74 $\pm$ 0.29 | 1.03 $\pm$ 0.06 | n.s.          | -                 | 0.200 $\pm$ 0.013 a | 0.173-0.226           | -             |
|                    | population mean | monophasic               | 12.54 $\pm$ 0.23 | 1.00 $\pm$ 0.09 | n.s.          | -                 | 0.216 $\pm$ 0.025 a | 0.167-0.265           | -             |
| 4-tert-octylphenol | 23%             | biphasic                 | 1.36 $\pm$ 0.30  | 1.74 $\pm$ 0.26 | 470 $\pm$ 440 | 5.789 $\pm$ 2.669 | 0.074 $\pm$ 0.037 a | 0.002-0.147           | 185 $\pm$ 51  |
|                    | 95%             | monophasic               | 29.39 $\pm$ 0.22 | 1.88 $\pm$ 0.13 | n.s.          | -                 | 0.124 $\pm$ 0.008 b | 0.108-0.141           | -             |
|                    | population mean | monophasic               | 12.74 $\pm$ 0.18 | 1.57 $\pm$ 0.11 | n.s.          | -                 | 0.102 $\pm$ 0.008 a | 0.086-0.118           | -             |

n.s. = no significant hormesis; CI = confidence interval; small letters indicate significant differences between  $ED_{50}$  values at  $\alpha=0.05$