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RESEARCH ARTICLE

Two organobromines trigger lifespan, growth, reproductive and transcriptional changes in *Caenorhabditis elegans*

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Abstract Organobromines of natural and artificial origin are omnipresent in aquatic and terrestrial environments. Although it is well established that exposure to high concentrations of organobromines are harmful to vertebrates, few studies have investigated the effect of environmentally realistic concentrations on invertebrates. Here, the nematode *Caenorhabditis elegans* was challenged with two organobromines, namely dibromoacetic acid (DBAA) and tetrabromobisphenol-A (TBBP), and monitored for changes in different life trait variables and global gene expression patterns. Fifty micromolar DBAA stimulated the growth and lifespan of the nematodes; however, the onset of reproduction was delayed. In contrast, TBBP changed the lifespan in a hormetic fashion, namely it was stimulated at 0.1 μM but impaired at 50 μM. The reproductive performance was even impaired at 2 μM TBBP. Moreover, DBAA could not reduce the toxic effect of TBBP when applied as a mixture. A whole-genome DNA microarray revealed that both organobromines curtailed signalling and neurological processes. Furthermore on the transcription level, 50 μM TBBP induced proteolysis and DBAA up-regulated biosynthesis and metabolism. To conclude, even naturally occurring concentrations of organobromines can influence the biomolecular responses and life cycle traits in *C. elegans*. The life extension is accompanied by negative changes in the reproductive behaviour, which is crucial for the stability of populations. Thus, this paper highlights that the effects of exposure to moderate, environmentally realistic concentrations of organobromines should not be ignored.

Keywords *C. elegans* · Organobromine · Microarray · Lifespan · Reproduction · Hormesis

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Introduction

Organohalogens, which include chlorine, bromine, iodine and fluorine compounds, gained most attention as water disinfection by-products, flame retardants and pesticides. However, naturally produced organohalogens are also of environmental relevance, a notion which is often ignored (Gribble 2010, 2012). At least 5,000 different naturally occurring halogenated organic compounds are known today, which are widely distributed throughout the terrestrial and aquatic environments (Asplund and Grimvall 1991; Gribble 2012). For example, elevated concentrations (up to 35 μg/l) of adsorbable organic bromine compounds (AOBr) were detected in German lakes (Putschew et al. 2003; Hütteroth et al. 2007). Given that the occurrence was recorded in late summer, the authors suggested that dead cyanobacterial cells may have released brominated compounds as well as haloperoxidases, which incidentally are responsible for the bromination of dissolved organic matter. Organobromines are known to be frequently produced as secondary metabolites of marine organisms, including macro- and micro-algae, bacteria, sponges and fungi

(Gribble 1999). In addition, Leri and Myneni (2012) recently described the path of inorganic bromine to carbon-bonded bromine by plants in terrestrial ecosystems. But what is known about the biological effects of these prevalent bromine compounds on ecosystems? Several detrimental characteristics (e.g. mutagenicity, carcinogenicity, spermatogenicity, endocrine disruptor activities and neurotoxicity) have previously been linked to organobromines (Linder et al. 1994; Birnbaum and Staskal 2004; Echigo et al. 2004). However, most studies utilised unrealistic concentrations (up to 2,000 mg/l), which were several magnitudes higher than the concentrations observed in real environments; even adsorbable organohalogen levels in industrial and hospital waste waters do not exceed 40 mg/l, and are frequently well below this concentration (Fürhacker et al. 2000; Kümmerer 2001; Shomar 2007). For example, 48 μg/l adsorbable organohalogens were found in an effluent of a Berlin wastewater treatment plant (Drewes and Jekel 1998) and Gellert (2000) determined concentrations from 10 to 1,370 μg/l in different wastewater effluents. An extensive study of 135 Swedish lakes and rivers showed that all sampled surface waters contain organohalogens, whereas concentrations between 11 and 185 μg Cl/l were measured (Asplund and Grimvall 1991). Moreover, organobromine concentrations up to 80 μg/l in drinking water after chlorination and 20 μg/l in raw water were detected (Manninen and Hasanen 1992). The use of excessive concentrations of organobromines may explain why previously described beneficial effects, including antitumor activities (Fuller et al. 1994; Linington et al. 2008), anti-bacterial and anti-inflammatory properties (Kuniyoshi et al. 1985; Gribble 2004) or simple hormetic effects (Wang et al. 2012) are often overlooked. Thus, there is a need to investigate the biological effects of exposure to organobromines at environmentally relevant concentrations.

In this study, the nematode *Caenorhabditis elegans* (*C. elegans*) was chosen as a model organism as it is amenable to detailed, yet rapid, ecotoxicological testing regimes (Leung et al. 2008). Two organobromine model substances, namely dibromoacetic acid (DBAA) and tetrabromobisphenol-A (TBBP), were chosen as test substances. Both organobromines and their derivatives are considered to be carcinogenic and toxic to vertebrates (Fukuda et al. 2004; Kuiper et al. 2007; Melnick et al. 2007) and are ubiquitously distributed in the environment (Hütteroth 2006; Covaci et al. 2009; Zhang et al. 2009). For instance, DBAA was detected in drinking water systems at concentrations ranging from 18 to 22 μg/l in the USA (Krasner et al. 2002) and 23–78 μg/l in Spain (Loos and Barcelo 2001). In hospital effluents, concentrations up to 58 μg/l were determined (Sun and Gu 2007). TBBP was reported as the brominated flame retardant with the highest production volume and concentrations between 4 and 180 μg/kg in sludge of Swedish sewage treatment plants and a mean of 25 μg/kg with peaks up to 9,800 μg/kg in English freshwater sediments were measured (Law et al. 2006). However, in vivo studies rarely employ realistic environmental concentrations and invertebrates, which play an important role in energy flows and nutrient cycling in freshwater ecosystems (Covich et al. 1999), are typically excluded. Therefore, we set out to explore crucial life trait variables, such as lifespan, stress resistance, reproduction and growth in organobromine-exposed animals. Moreover, a whole-genome DNA microarray was conducted to detect the underlying biomolecular mechanisms of the observed effects.

Materials and methods

Strains and conditions

The wild-type *C. elegans* strain N2 (var. Bristol) was maintained at 20 °C on nematode growth medium (NGM) seeded with the *Escherichia coli* feeding strain OP50 according to Brenner (1974). The N2 and the OP50 strain were obtained from the Caenorhabditis Genetics Center, University of Minnesota. DBAA and TBBP (Sigma-Aldrich, USA) were added to the NGM and the OP50 bacteria with final concentrations ranging between 0.1 and 50 μM. Equal amounts of solvent (final concentration of 0.3 % [*v*/*v*] DMSO; AppliChem, Darmstadt, Germany) were used in all conditions.

Lifespan assay

For each treatment 25 L4 larvae were transferred to NGM plates dosed with the test substances. About 150 L4 larvae per concentration of the following generation (F1) were transferred onto 10 plates with 15 larvae per plate. Surviving and dead animals were counted daily (starting at the first day of adulthood) until all individuals had died. Nematodes that failed to respond to contact stimuli were considered to be dead. Nematodes suffering from internal hatch and those that escaped from the NGM agar were censored. Adult nematodes were regularly transferred to new treatment plates. Statistical significance for alterations in the mean life span was calculated using a log-rank test (Bioinformatics at the Walter and Eliza Hall Institute of Medical Research; http://bioinf.wehi.edu.au/software/russell/logrank).

Thermal stress resistance and body length

About 30 L4 larvae (F1) per concentration and trial were randomly selected and transferred daily to fresh treatment plates. At the sixth day of adulthood, treated and untreated nematodes were moved either to 35 °C for 6.5 h and surviving and dead nematodes were counted thereafter (thermal stress test), or to 45 °C for 2.5 h and subsequently sized by means of a digital Microscope at 100-fold magnification (body length test).

Statistical significance was calculated via the chi-square test (thermal stress resistance) or Student's *t* test (body length) (SigmaStat 3.5; SPSS Inc., USA).

Reproduction assay

L4 larvae (F1) were transferred individually to treatment plates and moved to a fresh plate each day until reproduction was completed. The number of offspring per individual animal was determined daily and in total. In addition, the initial reproduction was recorded by transferring gravid animals (second day of adulthood (t_0)) of the parent generation to treatment plates for 30 min. The resultant offspring was separated onto small plates and the number of offspring counted after 75 h (from t_0). Statistical significance was calculated via the Student's *t* test (SigmaStat 3.5; SPSS Inc., USA).

Gene expression profiling

RNA preparation

Untreated young adult nematodes were treated with sodium hypochlorite (Sigma, Germany) according to Strange et al. (2007). To make sure that all eggs were handled in the same manner with this aggressive method, the pre-exposure of the worms was disclaimed. Thus, only one fraction of untreated eggs was isolated which was equally distributed to the substance plates. The obtained eggs were transferred to plates containing the respective doses of DBAA or TBBP and incubated at 20 °C. At the first day of adulthood, the nematodes were harvested by rinsing off with M9 buffer, washed at least three times with M9, shock frozen in liquid nitrogen and stored at −80 °C. For each condition, samples were cultivated in triplicate. The samples were milled with 0.5-mm glass beads in a homogeniser (SpeedMill PLUS, Analytik Jena, Germany) and thereafter the RNA was extracted using the innuSPEED Tissue RNA kit (Analytik Jena, Germany). The quality of the extracted RNA was examined by gel electrophoresis and the quantity with a spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). The RNA was stored at −80 °C and was used for the microarray as well as the quantitative real-time polymerase chain reaction (qRT-PCR) experiments.

DNA microarray

RNA was amplified with the MessageAmp™ Premier RNA Amplification Kit (Ambion, USA) which relies on the T7 in vitro transcription (IVT) amplification technology. First- and second-strand complementary DNA (cDNA) synthesis, complementary RNA (cRNA) synthesis, labelling, fragmentation, chip hybridization and scanning were performed according to the manufacturer's specifications (Affymetrix, USA). The whole-genome microarray chip (GeneChip *C. elegans* Genome Array, Affymetrix, USA) contains more than 22,500 transcripts from *C. elegans* and three chips were used per condition (DMSO-control, 0.1 and 50 μM DBAA as well as 0.1 and 50 μM TBBP). The quality and quantity of the RNA, cDNA and cRNA were assessed via capillary electrophoresis (Bioanalyzer 2100, Agilent Technologies, UK) and via spectrophotometry (NanoDrop 2000, Thermo Scientific, UK).

The transcription expression values were pre-processed with the MAS5 algorithm (Affymetrix Expression Console 1.2.0.20). After background correction, probe set summarization and CHP file generation, the expression signals were normalised using the relative median over all genes algorithm (BRB ArrayTools 4.3.0). Spots with spot intensities below the minimum signal value of 10 were excluded. Differentially expressed genes (DEGs) were identified with a random-variance *t* test and a significance analysis of microarrays (SAM) test. Genes were considered statistically significant if their *p* value was less than 0.05, the false discovery rate less than 0.3 and the fold change compared to control at least ≤0.67 or ≥1.5. To perform the GO Term analysis, the DEGs were processed with the Database for Annotation, Visualisation and Integrated Discovery (DAVID 6.7) (Huang et al. 2009a, b). Finally, the identified GO terms were visualised with QuickGO (Binns et al. 2009).

qRT-PCR

The Moloney Murine Leukaemia Virus (M-MLV) reverse transcriptase (Promega, USA) was used to synthesise the cDNA at 42 °C for 90 min. The quantitative real-time polymerase chain reaction (qRT-PCR) was performed with the Bio-Rad MyiQ iCycler (Bio-Rad, Germany) and the qRT-PCR Green Core Kit (Jena Bioscience, Germany). The efficiency of each primer pair, the primer sequences and the annealing temperatures are shown in Online Resource 1. All expression levels were normalised with the qRT-PCR values of the reference genes *act-1* and *cdc-42* and the quality of the PCR product was checked via gel electrophoresis. Significant changes (compared to the control) were determined by means of a Student's *t* test. Genes were considered statistically significant if their *p* value was less than 0.05 and the fold change compared to control at least ≤0.67 or ≥1.5. qRT-PCR was performed to verify the quality of the microarray data. The results are shown in Online Resource 2.

Results and discussion

Exposure to organobromines modulates lifespan but not stress resistance

Both organobromines were administered in five concentrations ranging from 0.1 μM (22 μg/l DBAA and 54 μg/l TBBP) to

50 μM (11 mg/l DBAA and 27 mg/l TBBP). Exposure to 50 μM DBAA extended the mean lifespan of nematodes by up to 10 % (Fig. 1a), whereas the maximum lifespan was not prolonged (Fig. 1b). In contrast, although TBBP provoked a life extension at the lowest concentration, exposure to the highest concentration shortened the lifespan of the nematodes (Fig. 1c). Numerous studies have previously described similar, so-called hormetic effects, where compounds exert a stimulatory effect at low concentrations but induce toxic effects at higher concentrations (Calabrese 2008). Interestingly, the life extension of 0.1 μM TBBP is also only visible in the mean and median lifespan, but the maximum lifespan is not affected (Fig. 1c, d). Because organobromines occur (in the environment) rarely in isolation, nematodes were also exposed to a mixture of the two organobromines. Since absolute measurements can vary between lifespan experiments, the single concentrations (0.1 and 50 μM TBBP as well as 50 μM DBAA) were repeated and compared to relevant mixture exposures. The first mixture, which combined the life-extending concentrations 0.1 μM TBBP and 50 μM DBAA, resulted in life prolongation (Fig. 2a, c). This effect seems to be additive compared to the effects of the single substances, whereas the significance limit was not reached here. In contrast, nematodes subjected to the second mixture, comprising 50 μM of each substance, did not benefit from the life-extending properties of DBAA (Fig. 2a, b). The lifespan of these nematodes was comparable to nematodes exposed to 50 μM TBBP.

Given the strong link between longevity and heat stress resistance (Johnson et al. 2002), it was perhaps surprising to observe that neither the single nor the mixed substances induced statistically significant changes in the thermal resistance test (Fig. 3). The absence of changes in thermal stress resistance might be due to the low sensitivity of this assay, since only one time point was measured. On the other hand, the connection between *C. elegans* lifespan and stress resistance remains uncertain, as exemplified by Muñoz and Riddle (2003) as well as Doonan et al. (2008) who showed that not all stress resistant nematodes are long lived. Moreover, Cypser et al. (2006) suggest that lifespan and stress resistance are driven by different molecular cues. Therefore, it seems reasonable to assume that thermal stress resistance and longevity are not linked in terms of DBAA and TBBP exposure.

Exposure to DBAA stimulates growth

Others have previously reported that the exposure to harmful, life-shortening chemicals can decrease the growth of *C. elegans* (Anderson et al. 2001; Roh et al. 2007; Wang et al. 2009). The disposable soma theory states that any additional energy required for life extension has to be subtracted from another sector, e.g., growth or reproduction (Kirkwood 1977, 1988), which was seen in *C. elegans* exposed to natural polyphenols (Saul et al. 2013). Interestingly, in the context of TBBP and DBAA exposures, neither the life-prolonging nor the life-reducing interventions led to shorter body lengths. Whilst TBBP did not influence the length at all, exposure to DBAA enhanced the growth at the higher concentrations (Fig. 4). These results are supported by several studies which have reported that life-prolonging conditions can promote the growth of invertebrates (Hofmann et al. 2012; Engert et al. 2013; Liu et al. 2013). The mixture exposure of 0.1 μM TBBP and

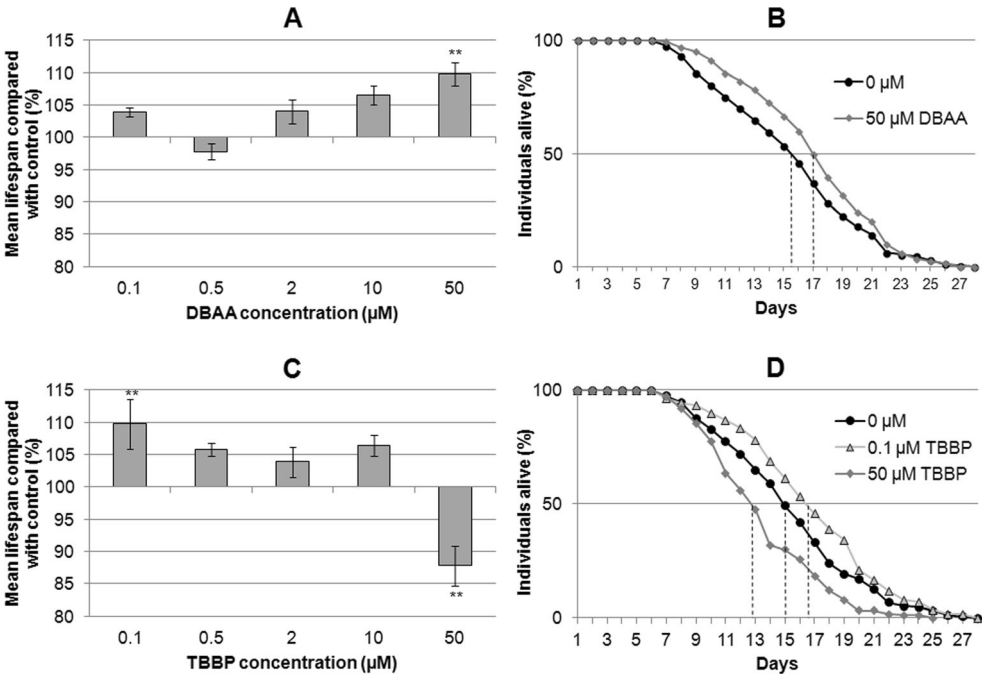

Fig. 1 The variation of lifespan. Shown is the percentage change of mean lifespan compared to the untreated control during exposure to (**a**) dibromoacetic acid and (**c**) tetrabromobisphenol-A as well as the Kaplan-Meier survival curves of 50 μM DBAA (**b**) and 0.1 and 50 μM TBBP (**d**) in comparison to the untreated control. Each bar represents the mean of two to four trials with a total of at least 192 nematodes per concentration. The errors represent the standard error of the mean and differences to the control are considered significant with $*p<0.05$ and $**p<0.001$

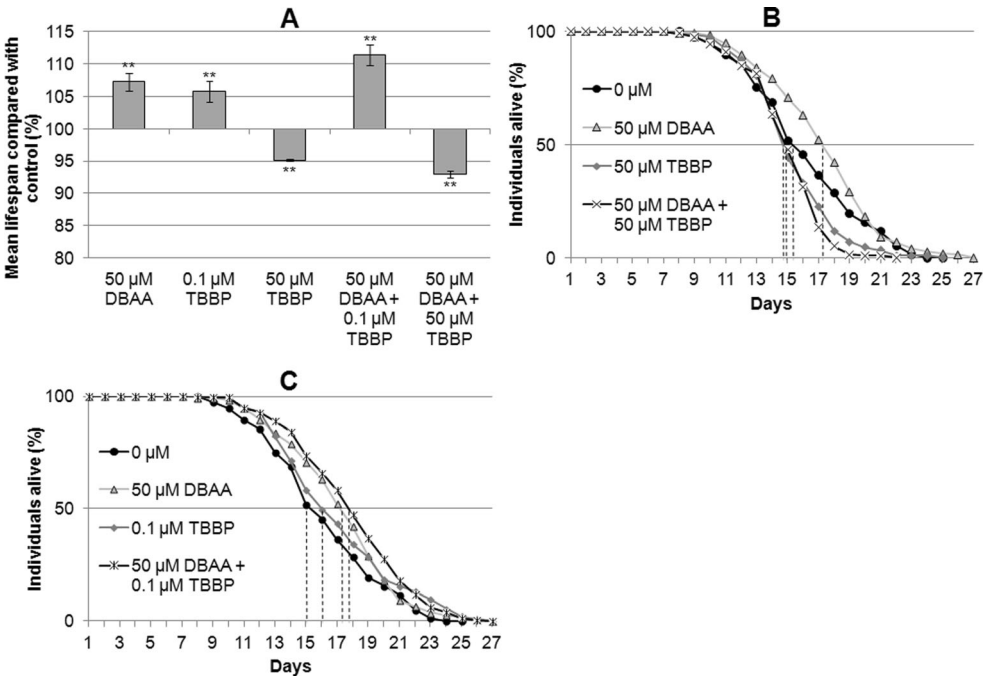

Fig. 2 Lifespan changes with DBAA/TBBP mixtures. Shown is the percentage change of mean lifespan compared to the untreated control during exposure to selected mixtures of DBAA and TBBP and the respective single concentrations (**a**). In addition, the survival curves of each mixture together with the single concentrations and the untreated control are shown (**b**, **c**). Each bar represents the mean of two to three trials with a total of at least 192 nematodes per concentration. The errors represent the standard error of the mean and differences to the control are considered significant with *$p<0.05$ and **$p<0.001$

50 µM DBAA did not enhance the growth further (compared to DBAA alone), and the 50 µM DBAA and 50 µM TBBP mixture resulted in growth stimulation which was in between the two respective single dose exposures (Fig. 4).

Both organobromines inhibit reproduction in different ways

Reproductive fitness is, in terms of maintaining a population, one of the most sensitive targets of toxicants. Indeed, some life-prolonging substances have been found to inhibit reproductive output or timing (Harrington and Harley 1988; Saul et al. 2013). However, exposure to DBAA did not change reproductive fitness in terms of daily or total number of offspring produced (Fig. 5a). Thus, there is no direct evidence of energy reallocation in DBAA treated nematodes, which (according to the disposable soma theory) could have explained the longevity effect. In contrast, TBBP had a more pronounced toxic effect on reproduction, where even low concentrations (2 µM TBBP) significantly reduced the daily and total brood size (Fig. 5b). Furthermore, the toxicity of TBBP cannot be mitigated by DBAA (Fig. 5c).

The reproductive timing is crucial to any population (Roff 2001), as in nature, many animals do not live until their reproductive capability has peaked. A concentration of 50 µM DBAA (in isolation or as part of the mixture) was able to delay the onset of reproduction (Fig. 5d), the exposure

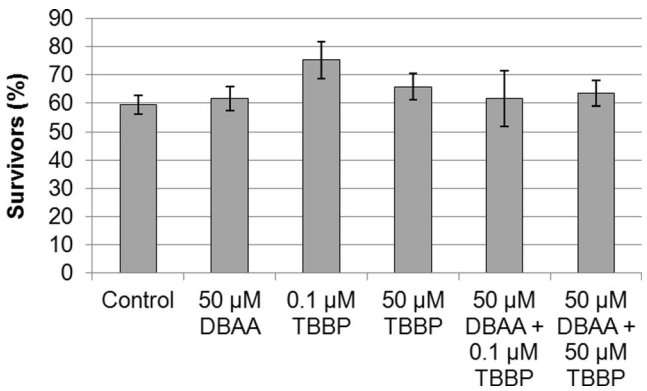

Fig. 3 The influence of DBAA and TBBP on thermal stress resistance. At the sixth day of adulthood, dead and alive animals were scored following a 6.5 h exposure 35 °C. The bars display the mean number of survivors in percent from three independent trials. At least 106 nematodes per concentration were scored. The errors represent the standard error of the mean and no variation reached the significance level of $p<0.05$

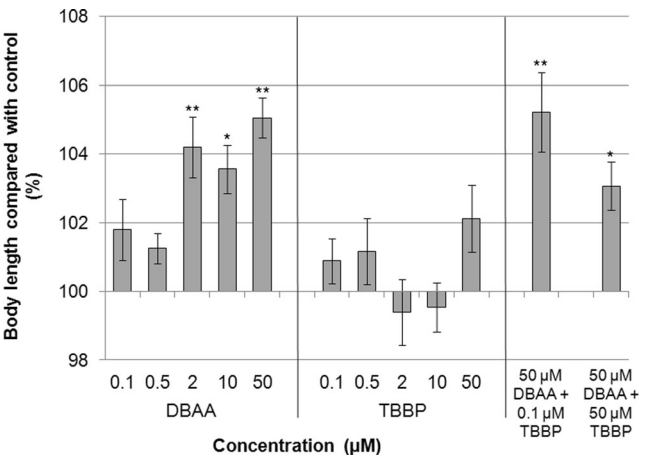

Fig. 4 Body length of nematodes exposed to organobromines at the sixth day of adulthood. Shown is the average of three to five trials with 126-188 sized nematodes per concentration in total. The errors represent the standard error of the mean and differences to the control are considered significant with *$p<0.05$ and **$p<0.001$

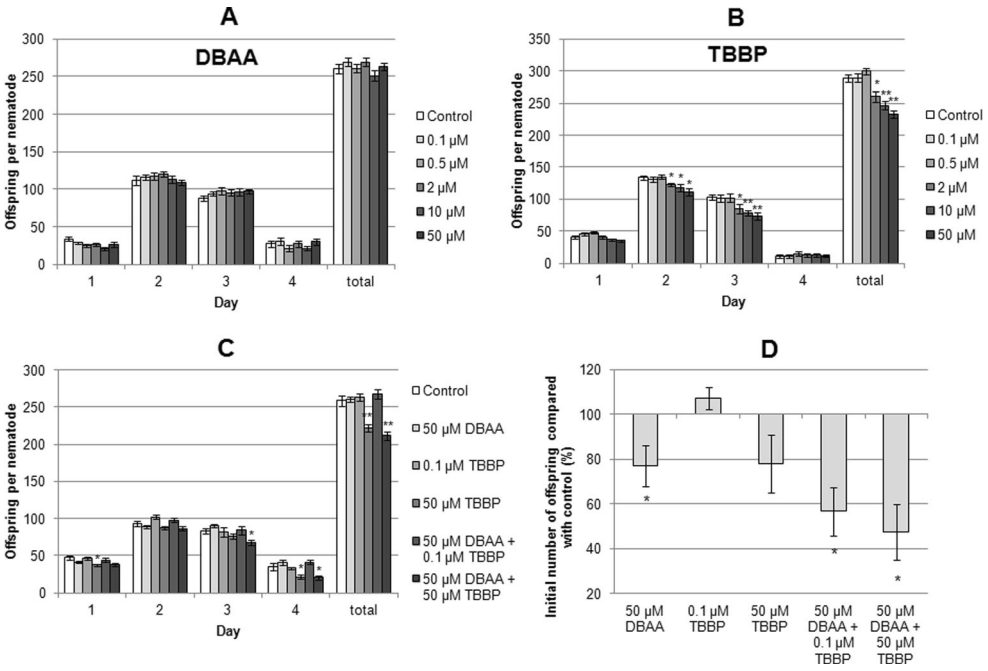

Fig. 5 The impact of organobromines on reproduction. The number of offspring per day and in total during exposure to DBAA (**a**), TBBP (**b**) and the mixtures (**c**) were determined in three independent trials with a total of 27–30 nematodes per concentration. The first day refers to the first day of adulthood. Moreover, the initial reproduction after 75 h (after the egg stage) was counted in two independent trials with a total of 20 nematodes per concentration (**d**). The errors represent the standard error of the mean and differences to the control are considered significant with $*p<0.05$ and $**p<0.001$

to 50 µM TBBP inhibited the onset of reproduction by trend, and the most pronounced effect was observed when nematodes were subjected to both organobromines (although it should be noted that the differences between the treatments were not deemed to be statistically significant).

If the delay in the onset of reproduction is due to a delay in development, it may contribute to the observed longevity effect. This is plausible, since developmental and lifespan regulation share some important molecular pathways (Gerisch et al. 2001; Jia et al. 2004). Moreover, it should be noted that the increased body length could also be an artefact due to a delay in development. Since *C. elegans* tend to shrink with age, a developmental retardation triggered by DBAA could lead to a delay in shrinking. However, the reproductive delay due to 50 µM DBAA is only visible when the worms were synchronised in the egg stage (Fig. 5d) and not in the L4 stage (Fig. 5a). Thus, the main developmental delay seems to be limited to the embryonic development, which is negligible for the growth and lifespan assays since the nematodes were synchronised in the L4 stage.

Gene expression during chronic exposure to organobromines

Whole-genome *C. elegans* microarrays were performed on samples derived from young adult nematodes exposed to DMSO (i.e., the control group) or to the organobromines (0.1 and 50 µM DBAA or 0.1 and 50 µM TBBP). It should be noted that, in contrast to the life history trait assays, the parental generation was not pre-exposed to the substances but nematodes were transferred directly as eggs to the substance plates. It cannot be excluded that this different handling could have an influence on the gene expression data.

In total, 349 (50 µM DBAA), 102 (0.1 µM DBAA), 299 (50 µM TBBP) and 88 (0.1 µM TBBP) transcripts were differentially expressed (compared to control), and perhaps surprisingly more genes were down-regulated than up-regulated in all treatment groups (Fig. 6). This may be due to the relatively long exposure duration of 3 days. The induction of stress-responsive genes often occurs immediately after stress exposure, but is typically timely limited and is followed by down-regulations and

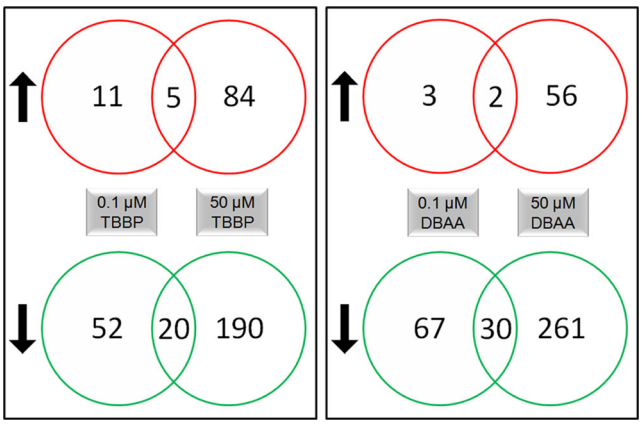

Fig. 6 Venn diagram of differentially expressed genes. The amount of up-regulated (*upper diagrams*) and down-regulated (*lower diagrams*) genes compared with control after exposure to two concentrations of TBBP (*left*) and DBAA (*right*) are displayed. Genes were considered statistically significant if $p<0.05$ and a fold change of at least ≤0.67 or ≥1.5, respectively, was reached. The raw microarray data are shown in Online Resource 6

negative feedback loops (de Nadal et al. 2011). Although it is likely that only few stress defence and repair genes remain up-regulated following long exposure duration, it is this chronic exposure which reflects the real environmental situation. Indeed, most stressors do not disappear suddenly and animals in aquatic environments often do not have the chance to escape.

Induction of proteolysis and repression of signalling during TBBP exposure

The gene ontology (GO) analysis of genes that were down-regulated after 50 μM TBBP exposure highlighted the presence of a diminished stimuli transfer (Table 1; the full list and graph of DBAA- and TBBP-related GO terms are shown in

Table 1 Selected GO terms of differentially expressed genes during TBBP exposure

	down-regulated genes after 50 μM TBBP exposure	down-regulated genes after 0.1 μM TBBP exposure	up-regulated genes after 50 μM TBBP exposure
BP	ion transport		
	neurological system process		
	cognition		
	sensory perception of chemical stimulus		
	response to ER stress		
	ER-nuclear signalling pathway		
	ER unfolded protein response		
CC	integral to membrane		integral to membrane
	intrinsic to membrane		intrinsic to membrane
	integral to plasma membrane		
	intrinsic to plasma membrane		
	cell part		
MF	receptor activity		
	signal transducer activity		
	molecular transducer activity		
	ion transmembrane transporter activity		
	substrate-specific transmembrane transporter activity		
	neurotransmitter receptor activity		hydrolase activity
	neurotransmitter binding		metalloendopeptidase activity
	passive transmembrane transporter activity		endopeptidase activity
	transmembrane receptor activity		
	gated channel activity		
	extracellular ligand-gated ion channel activity		
	substrate specific channel activity		

The up-regulated genes after 0.1 μM TBBP exposure yielded no significant changed GO terms

BP biological process, *CC* cellular component, *MF* molecular function, *TBBP* tetrabromobisphenol-A, *ER* endoplasmic reticulum

Online Resources 3 and 4). In detail, down-regulated genes during TBBP exposure were assigned to GO terms such as 'signal transducer activity', 'substrate-specific transmembrane transporter activity' and 'neurotransmitter receptor activity'. The temporal restriction of stress response is important for viability, and this is achieved by down-regulation of the respective signalling mechanisms (de Nadal et al. 2011), thus, it can be assumed that stimuli, like the stress stimuli from TBBP itself, were less processed and the typical stress response cascades were hardly induced. Similar gene repressions were also detected with chronic bromopropane exposure in rats (Mohideen et al. 2009). Moreover, some general stress-responsive genes were down-regulated, including *hsp-70* and *hsp-16.41* (Table 2: only genes assigned with a gene name are shown here; the full list of DEGs and their fold change are shown in Online Resource 5) which act as chaperones to

Table 2 Selected differentially expressed genes during organobromine exposure

	Down-regulated						Up-regulated	
50 μM TBBP	acp-3	fbxa-46	mbr-1	plx-1	srh-109	srw-59	abt-5	lbp-8
	alh-2	fbxa-47	mpz-1	pqn-66	srh-182	srw-67	clec-136	lipl-2
	aptf-1	gbb-1	ncx-9	pqn-73	srh-211	srx-6	clec-46	mec-10
	arrd-21	gck-4	nhr-15	sdz-19	srh-295	srx-67	cnp-2	nas-12
	asp-7	glb-4	nhr-156	sdz-3	srh-82	srxa-7	col-37	ndx-7
	best-8	gpa-18	nhr-192	ser-1	srh-87	str-109	col-58	nep-5
	ccpp-6	grd-12	nhr-9	sfa-1	sri-10	str-115	cutl-2	npr-6
	clec-185	grl-14	oac-3	smf-3	sri-24	str-119	cyp-13A4	pgp-1
	clec-40	hpo-11	oac-31	snx-3	sri-65	str-130	cyp-13A5	srh-214
	csp-2	hsp-16.41	oac-53	sra-14	srsx-13	str-15	glb-2	srsx-37
	cyp-13A7	hsp-70	ocr-2	sra-26	srsx-32	str-190	grl-10	srt-72
	eyg-1	lgc-1	ocr-3	srbc-81	sru-39	str-227	grl-29	srw-73
	fbxa-213	lgc-18	pbo-6	srbc-9	srv-19	tag-138	kqt-3	unc-30
	fbxa-22	lgc-40	pix-1	sre-42	srv-7	tag-272	lad-2	
		lipl-8	pkc-2	srg-7	srw-15	tpxl-1		
						unc-17		
0.1 μM TBBP	amt-2	clec-234	lgc-1	oac-45	sfa-1	sri-24	clec-157	srd-16
	ceh-23	cyp-13A7	nhr-216	ocr-3	srab-6	sri-61	col-37	srd-19
	clec-101	dos-2	nhr-250	osr-1	srbc-9	srw-129	cutl-13	srj-16
	clec-159	egl-19	nhr-265	ram-5	sre-44	str-211	nhr-195	unc-30
	clec-18	gab-1	nstp-5	rbbp-5	srg-7	str-81		
	clec-185	hex-3	oac-18	rbc-2	srh-231	tag-138		
				scl-14	srh-87	tra-1		
50 μM DBAA	acr-7	gar-3	nhr-119	sra-37	srj-13	str-48	clec-157	nhr-238
	ant-1.2	gbb-1	nhr-150	srab-6	srsx-18	str-63	clec-58	phy-3
	arrd-21	gcy-34	nhr-192	srb-13	srsx-7	str-84	col-186	plc-1
	btb-18	gei-1	nhr-196	srbc-1	srt-55	sulp-3	col-33	srh-49
	cdh-8	gnrr-6	nhr-199	srbc-24	srt-69	tag-341	fbxa-165	srj-16
	ceh-14	grk-1	nlp-2	srbc-40	sru-39	tbc-19	fbxb-31	srt-72
	clec-101	gsa-1	npr-18	srbc-57	srv-19	ttx-3	fbxb-33	srw-61
	clec-18	gst-33	npr-19	srd-56	srw-10	twk-16	grl-10	str-214
	clec-243	haf-8	npr-26	sre-29	srw-137	twk-45	hen-1	tpa-1
	clec-76	hda-4	ntl-9	sre-47	srw-47	twk-6	hlh-31	unc-70
	clec-8	hlh-30	old-1	sre-53	srw-66	ugt-54	nhr-195	
	col-85	hpo-11	olrn-1	srg-24	srx-111	unc-17		
	cyp-13A7	ins-24	osr-1	srh-180	srx-53	unc-34		
	dat-1	jud-4	pbo-6	srh-270	srx-90	unc-7		
	eel-1	klp-13	pqn-10	srh-295	str-116	uvt-3		
	egl-19	lgc-18	pqn-62	srh-308	str-120	wht-5		
	ets-5	lgc-33	smf-3	srh-87	str-182	wrt-7		
	fbxa-213	lips-6	spp-11	sri-28	str-229	ztf-2		
	fkh-7	math-16	spp-6	sri-46	str-250			
	flp-27	math-28	spsb-1	sri-61				
0.1 μM DBAA	best-8	cyp-13A7	nhr-118	rgs-4	srb-12	srw-23	col-102	
	ceh-14	fut-4	nhr-119	scl-14	srbc-52	srw-53	col-37	
	chil-20	hda-4	oac-18	scl-24	sre-47	srw-79	srd-16	
	clec-18	hlh-30	oac-56	sfa-1	sre-53	sto-6	srj-16	
	clec-185	lin-14	pbo-6	smf-3	srh-87	str-114	unc-70	
	clh-2	lipl-8	pqn-62	sra-26	sri-63	str-204		
	col-53				sru-8	tag-336		

handle misfolded and unfolded proteins. However, certain degradation mechanisms (e.g. 'hydrolase activity' and 'endopeptidase activity') were up-regulated, probably to remove TBBP-generated damaged proteins. The increase in protein degradation processes was shown to be a clear sign of oxidative stress (Pacifici and Davies 1990) which is in line with the observed toxic action of TBBP regarding lifespan and reproduction. Moreover, the transmembrane transporter *pgp-1* (P-glycoprotein (PGP) related) was induced (Table 2 and Online Resource 5). PGPs are involved in the multidrug resistance phenomena and are also known to protect the cells of *C. elegans* from harmful substances (Broeks et al. 1995, 1996; James and Davey 2009). In addition, it was assumed that PGPs are biomarkers of pollution since they are inducible by organic xenobiotics as shown in oysters (Keppler and Ringwood 2001) or aquatic invertebrates (Smital et al. 2000) and which is reviewed in detail by Bard (2000). Whether the induction of *pgp-1* is correlated with an increased transport of TBBP or its metabolites through the cell membrane is unclear, however.

Recently, 50 μM TBBP was shown to elicit neurotoxic effects in *C. elegans*, impacting the locomotive behaviour, the defecation frequency and chemical sensory system (Ju et al. 2014). These findings are also mirrored in the expression patterns, where genes involved in several neurological relevant GO terms were shown to be down-regulated (Table 1). Even the reproductive effects (Fig. 5) were pinpointed at the molecular level, since several reproduction-relevant genes were down-regulated. For instance, *glb-4* (globin (GLB) related) was repressed with a fold change of 0.45 (Online Resource 5) compared to control. GLB-4 is required for embryonic viability, fertility and vulva development, thus the down-regulation of *glb-4* may lead to a noticeable impact on reproductive performance.

Interestingly, some signalling cascades were also down-regulated at the lower (0.1 μM) TBBP exposure, but the up-regulation of the proteolytic mechanisms or of *pgp-1* were not observed. This might be an indicator for mild (or reduced) stress or indeed hormesis, as seen in the lifespan assays (Fig. 1).

Increased metabolism and decreased signalling during DBAA exposure

Exposure to either of the DBAA concentrations also led to the repression of several neurological, behavioural and signalling related genes (Table 3). However, as 50 μM TBBP and 50 μM DBAA elicited different effects in almost all life trait variables of *C. elegans* (Figs. 1, 4 and 5), it is not unreasonable to assume that the respective transcriptional responses will be distinct. Indeed, a detailed comparison of all down-regulated genes (Online Resource 5) revealed that only 41 of the 291 repressed genes during 50 μM DBAA exposure were also down-regulated during 50 μM TBBP exposure. Despite the similarity of the overall GO terms, the differences observed in the life history traits and the identity of the differentially expressed genes support the notion that the organobromines trigger different, unique responses. Moreover and in contrast to TBBP, the exposure to DBAA induced metabolism and biosynthetic processes, but not proteolytic processes. This may account for an enhanced overall body fitness of DBAA treated nematodes, resulting in the observed increase in growth. The metabolic and biosynthetic increases could also be a sign of retarded ageing, since ageing is accompanied by a decline of the metabolic rate and repression of genes involved in biosynthesis and metabolism (Lee et al. 1999; Braeckman et al. 2002).

The differences between DBAA and TBBP exposures were also apparent in neurotoxicology assays (Ju et al. 2014). Whilst TBBP exerted a negative impact on neurotoxicological markers, the exposure to DBAA was, in some cases, neurostimulatory, including the locomotive behaviour and the pharyngeal pumping frequency. It remains unclear how these neurostimulative effects harmonises with the down-regulation of genes involved in neurogenesis, neurological system processes and signalling. Maybe the up-regulation of some other neurological relevant genes, like *hen-1* (HEsitatioN behaviour) and *unc-70* (UNCoordinated) (Table 2), are superior to the neurological gene repressions. However, this speculation needs to be confirmed with further tests.

Interestingly, even at phenotypically ineffective concentrations of DBAA (0.1 μM), a down-regulation of signalling and neurological relevant genes was observed, highlighting that the molecular response to DBAA is more sensitive than the organismal and behavioural endpoints.

Outlook

The determined gene expression patterns do not necessarily reflect the protein quantity or functionality since this also depends on mRNA stability and posttranscriptional or post-translational regulations and modifications. Using the example of plant abiotic stress, Hirayama and Shinozaki (2010) showed how complex and multifaceted the stress response can be. Another convincing example was shown by Gellner et al. (1992) who reported that the transcription of *hsp70.1* in two *Hydra* species was similarly induced by heat shock; however, the mRNA was not stable in *Hydra oligactis*. This was assumed to be the reason for the inability of this species to induce thermotolerance after heat shock (Bosch et al. 1988). Thus, the quantities of the corresponding proteins of the induced or repressed transcripts should be determined in addition. Other important factors in gene regulation are the microRNAs, which were not covered by the microarray performed in this study. MicroRNAs are crucial in gene

Table 3 Selected GO terms of differentially expressed genes during DBAA exposure

	down-regulated genes after 50 μM DBAA exposure	down-regulated genes after 0.1 μM DBAA exposure	up-regulated genes after 50 μM DBAA exposure	up-regulated genes after 0.1 μM DBAA exposure
BP	sensory perception of chemical stimulus		regulation of gene expression	
	cognition		regulation of transcription, DNA-dependent	
	neurogenesis		regulation of nitrogen compound metabolic process	
	generation of neurons		regulation of cellular metabolic process	
	neurological system process		regulation of macromolecule biosynthetic process	
	regulation of response to stimulus	nervous system development	regulation of cellular biosynthetic process	
	regulation of feeding behaviour		regulation of primary metabolic process	
	regulation of pharyngeal pumping		regulation of macromolecule metabolic process	
	thermosensory behaviour		regulation of RNA metabolic process	
	regulation of behaviour			
	cell surface receptor linked signal transduction			
	amine transport			
	G-protein coupled receptor protein signalling pathway			
	protein modification process			
CC	integral to membrane			
	intrinsic to membrane			
MF	signal transducer activity		transcription regulator activity	structural molecule activity
	molecular transducer activity			structural constituent of cuticle
	receptor activity			
	transmembrane receptor activity			
	amine transmembrane transporter activity			

BP biological process, *CC* cellular component, *MF* molecular function, *DBAA* dibromoacetic acid

regulation (Zhang et al. 2007) and more than 200 were identified in *C. elegans* so far with several having distinct roles in lifespan, stress response and development (Abbott 2011). A microRNA-microarray could detect hidden regulative processes in response to organobromines. Not least, to understand the action of organobromines, metabolomic studies should be addressed. These approaches could give answers about the metabolism of the compounds itself as well as about the metabolic changes in the organism due to substance exposure and will provide an important step for the understanding of chemico-biological interactions (Prince and Pohnert 2010; Putri et al. 2013).

Conclusion

Exposure to 50 μM DBAA stimulated the lifespan and growth of *C. elegans*, without impacting the total reproductive performance. However, the onset of reproduction was delayed, which can have a severe impact for populations in nature. Surprisingly, the life extension was not accompanied by an enhanced thermal stress resistance. Other stress parameters (e.g. oxidative or UV stress) should be tested to explore in more detail the potential protective effects of DBAA. Moreover, a more extensive concentration range should be tested to exclude or verify a possible hormetic action.

In contrast, TBBP changed the lifespan in a hormetic manner. Whilst exposure to low doses of TBBP (0.1 μM) resulted in a life extension without any apparent side effects in the life trait variables tested, the higher dose (50 μM TBBP) shortened the lifespan. Moreover, TBBP had an impact on reproductive output (even at low concentrations) and delayed the onset of first reproduction by trend. Thus, the assumption that organobromines in moderate concentrations are stimulatory in *C. elegans* is only partly valid. Furthermore, the detrimental effects following an exposure to 50 μM TBBP could not be moderated by the addition of 50 μM DBAA. In fact, the mixture exposure delayed the reproductive timing further.

Both organobromines led to a down-regulation of signalling cascades and neurological system processes, probably in order to inhibit energetically expensive stress defence mechanisms during chronic substance exposure. The harmful effect of 50 μM TBBP was manifested via the induction of proteolytic activities, in contrast exposure to 50 μM DBAA upregulated metabolic and biosynthetic processes.

To conclude, even at relatively moderate concentrations and disregarding the lifespan extension effects, the results presented here suggest that organobromines have the potential to change the dynamics of populations, especially by influencing the neurological and reproductive processes. In short, exposure to organobromines can have unpredictable consequences for populations.

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