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PHYSIOLOGICAL MODES OF ACTION OF TOXIC CHEMICALS IN THE NEMATODE *ACROBELOIDES NANUS*

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Abstract—To gain a better understanding of the mechanisms through which a chemical exerts toxicity, a deeper insight is needed regarding the physiological processes that take place during a toxic stress. This issue can have important benefits for risk assessment, because it can contribute to a better interpretation of toxicity data. Here, we study the physiological mode of action of three different compounds (cadmium, carbendazim, and pentachlorobenzene) with an experimental data–based approach using whole life-cycle toxicity data from the nematode *Acrobeloides nanus*. We use a process-based model, based on the dynamic energy budget theory, to study the fluxes of energy related to physiological processes and their variation throughout the life cycle. With this approach, we unravel the physiological modes of action based on resource allocation, and we model the effects of the different modes of action at the population level. The mode of action of carbendazim was through a decrease in assimilation, with an additional effect on the production of reactive oxygen species (ROS). Cadmium increased the costs of growth, with an extra effect on ROS production, and pentachlorobenzene decreased assimilation. We compared the present results with those of previous studies using the nematode *Caenorhabditis elegans*, and we found that the modes of action for the three compounds differed from those found in *A. nanus*, showing that the life-history characteristics of each organism have a clear influence on the resulting modes of action. This highlights the importance of the interactions between a chemical and the biological characteristics of the organism in determination of the resulting physiological modes of action.

Keywords—Physiological mode of action *Acrobeloides nanus* Toxic mechanisms Dynamic energy budget

INTRODUCTION

Current methods for classifying chemicals by their mode of action generally can be based either on experimental data or on structure. Experimental data–based methods assess a chemical's mode of action by analyzing the data from toxicity tests, whereas structure-based approaches classify compounds based on their functional groups. A widely used structure-based classification was developed by Verhaar et al. [1], who assigned chemicals to one of four classes: Narcotics, polar narcotics, reactive chemicals, and specifically acting chemicals with receptor-mediated toxicity. This classification is derived from the structural characteristics of the compounds, with the underlying assumption that chemically similar compounds have similar biochemical modes of action, which often, but not necessarily, is the case. The activity of a toxicant in an organism depends on physical, chemical, and biological factors, among which interactions also may exist, making determination of the mechanism of toxic action a difficult task [2]. To gain a better understanding of the mechanisms through which a chemical exerts toxicity, a deeper insight is needed regarding the physiological processes that take place during a toxic stress. The physiological mode of action may vary depending on the species, compound, and endpoint being studied. Consequently, studies that provide a deeper insight regarding these mechanisms can become important tools for risk assessment, contributing to a better interpretation of toxicity data.

In the present study, we examine the physiological mode of action of three different compounds: Cadmium (heavy metal), carbendazim (DNA synthesis inhibitor [3]), and pentachlorobenzene (narcotic [4]). We take an experimental data–based approach that uses whole life-cycle toxicity data from different endpoints. The present study illustrates how physiological modes of action can be analyzed from a mechanistic perspective based on the chemical's effects on the organism's energy budget. This is done using a process-based model that studies the fluxes of energy, related to physiological processes, and their variation throughout the whole life cycle. This model is based on the dynamic energy budget (DEB) theory, which describes the functioning of individual organisms based on a set of rules for metabolic organization [5,6]. Exposure to toxicants is regarded as a change in energetic parameters, such as an increase in maintenance costs or a decrease in assimilation of energy from food. This insight inspired the development of DEBtox [7], a suite of models used to analyze toxicity experiments in a process-based manner. The analysis reveals the physiological mode of action based on the changes in energy allocation patterns that occur in response to the chemical. In the DEB scheme, part of the food is assimilated, contributing to the reserves, and the rest is transformed into feces. These resources are distributed in a fixed fraction between somatic growth/maintenance and reproductive output/maturation. Within this scheme (Fig. 1), a chemical may exert a toxic effect on different energetic parameters: Decreasing assimilation, increasing costs of maintenance, increasing costs of growth, increasing costs of reproduction, or posing a direct hazard to the embryo [7]. The dissection of the physiological mode of action of toxic compounds is interesting from a scientific viewpoint, because it provides an insight regarding the physiological processes that occur during a toxic stress and how these processes are reflected on the different endpoints (survival, reproduction, and growth). Additionally, under-

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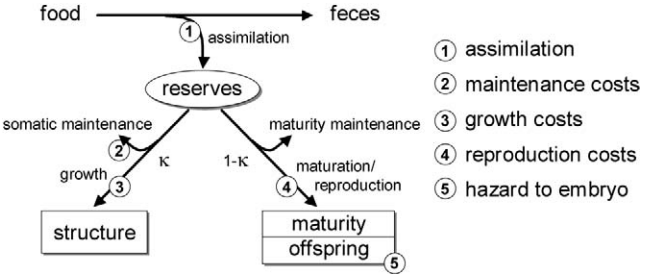

Fig. 1. Dynamic energy budget scheme and target parameters under which a compound can exert its toxic mode of action.

standing the details about the mode of action of toxicants is essential for the extrapolation to population-level effects.

The DEBtox method has been extended recently to model effects at the population level by means of simultaneously fitting data from the different life-history traits [8]. In this way, the effects on the different endpoints are integrated into a single parameter, the population growth rate, which provides a measure of ecological impact that is more relevant than any isolated endpoint [9]. Furthermore, because other factors, such as temperature or food limitation, have predictable consequences in DEB theory [10], their effects on the population growth rates in combination with increasing concentrations of toxicant can be modeled.

The DEBtox method was used in the present paper to model simultaneously the effects on survival, growth, and reproduction of three different compounds (cadmium, pentachlorobenzene, and carbendazim), to elucidate their physiological toxic mode of action, and to reveal how these modes of action affect the population growth rates in the nematode *Acrobeloides nanus*. This nematode is a parthenogenetic species with an average life span of 50 to 60 d, placing it in the category of intermediate-life-span nematodes. This species was selected for the experiments with the aim of comparing the present results to those obtained in previous studies with the nematode *Caenorhabditis elegans* [11,12], which is a facultative, self-fertilizing hermaphrodite that also can reproduce sexually in the presence of males and has a shorter life span (~20 d).

MATERIALS AND METHODS

Chemicals

Pentachlorobenzene was purchased from Aldrich (purity, 98%; Steinheim, Germany). An initial pentachlorobenzene solution of 10 mg/ml ethanol was prepared. Nominal pentachlorobenzene concentrations used for the experiments ranged from 50 to 130 mg/L agar. An ethanol blank was used as the control.

Carbendazim was purchased from Riedel-de-Haen (purity, 99%; Seelze, Germany). An initial carbendazim solution of 300 mg/L ethanol was prepared. Nominal carbendazim concentrations used for the experiments ranged from 0.15 to 1.5 mg/L agar. An ethanol blank was used as the control.

Cadmium chloride ($CdCl_2$) was purchased from Merck (purity, >99%; Schuchardt, Germany). An initial $CdCl_2$ solution of 1 mg/ml water was prepared. Nominal $CdCl_2$ concentrations used ranged from 2 to 12 mg/L agar.

Acrobeloides nanus

Nematodes were extracted with an Oosterbrink elutriator [13] from soil samples that were collected from an experimental field at Bovenbuurt (3 km north-northeast of Wageningen, The Netherlands). From the obtained water suspension, *A. nanus* individuals were classified to the species level and placed on Petri dishes to start populations. Populations were maintained at 20°C.

Culturing

All nematodes were reared on nematode growth medium agar plates seeded with the OP50 strain of *Escherichia coli* as a food source [14,15]. Stock cultures of OP50 were stored at −80°C, and the bacterial cultures were grown in autoclaved Luria Broth medium (10 g of peptone, 10 g of yeast extract, and 5 g NaCl/L water) for 16 h at 37°C and 150 rpm.

All experiments were started with a first synchronization in which gravid adults were transferred to 6-cm Petri dishes and allowed to lay eggs for a period of 4 to 8 h, after which they were removed. These eggs were allowed to hatch and develop into gravid adults, with which a second synchronization was performed, this time on agar containing the corresponding concentration of toxicant. In this way, we obtained the study individuals, which were exposed throughout their whole life span (since the egg stage) to the experimental treatments.

In the case of carbendazim, two experiments were carried out. One was performed as described above, with carbendazim concentrations from 0.15 to 0.75 mg/L agar. A second one was carried out in which all individuals were allowed to hatch under control conditions and then transferred to their respective carbendazim concentrations (0.3–1.5 mg/L agar). This is because this compound dramatically inhibits hatching, so experiments performed after the egg stage can be carried out only with very low concentrations of this compound, giving way to very low sublethal effects. By allowing the individuals to hatch under control conditions and then transferring them to the contaminated dishes, we were able to use higher concentrations and, thus, to observe larger sublethal effects.

Reproduction and survival

The newly hatched nematodes were transferred to individual wells (12-well plates from Greiner Bio-One, Kremsmuenster, Austria) for observation. Each well contained 2 ml of agar with the corresponding concentration of toxicant. The nematodes were transferred to a new well daily to count the number of offspring per individual. Reproduction was recorded in 20 to 40 individuals for each concentration. Death was scored when observing the absence of pharyngeal pumping and no movement in response to touch. Survival data were recorded in 20 to 40 individuals per treatment.

Growth

Pictures were taken of 8 to 10 individuals per treatment with a Cool Snap camera (Photometrics, Tucson, AZ, USA) at intervals of 6 h at the beginning of the growth curve and at longer intervals toward the end. The pictures were digitized with the Image Pro Express 4.0.1 software package (Media Cybernetics, Silver Spring, MD, USA) to obtain length and area measurements of the nematodes at the different time points.

Modeling

The DEBtox method [7] was used to analyze the life-history traits of *A. nanus* and the effects of the three different toxicants on these traits. In this approach, the internal concentration causes the effects; therefore, the first step in the model chain

Table 1. Parameters used in the models[a]

	Cadmium	Pentachlorobenzene	Carbendazim	
			Experiment 1	Experiment 2
Animal parameters				
Von Bertalanffy growth rate (1/d)	0.314 (0.277–0.332)[b]	0.269 (0.235–0.293)	0.388	0.393
Initial length (μm)	18.5 (NE)	18.5 (NE)	18.5 (NE)	23.7
Length at which ingestion is half of maximum (μm)	22.1 (21.8–22.4)	20.1	25.2	26.3
Length at start egg production (μm)	47.9 (46.9–48.6)	46.2 (44.8–47.5)	47.8	48.2
Maximum length (μm)	62.7 (61.9–64.0)	63.5 (62.0–65.2)	72.5	66.0
Maximum reproduction rate (eggs/d)	35.1 (32.7–37.0)	37.0	37.5	57.4
Aging parameters				
Damage killing rate (1/d)	0.00128 (8.52e-4–1.83e-3)	0.00157	0.00160	0.00142
Damage tolerance on reproduction [−]	18.1 (15.3–20.4)	16.0	14.0	15.1
Toxicity parameters				
Elimination rate (1/d)	0.0295 (0.0265–0.0320)	10 (NE)	10 (NE)	10 (NE)
No-effect concentration (NEC) for survival, based on receptor occupation [−]	0.01 (NE)	0.01 (NE)	0.01 (NE)	0.01 (NE)
Killing rate based on receptor occupation (1/d)	0.271 (0.0595–0.874)	0.177 (0.0840–0.636)	0.978	0.970
Receptor knock-out rate (mg/L/d)	0.0104 (4.06e-3–0.0589)	0.000641	0.00501	0.00536
NEC for effects on growth/reproduction (mg/L)	5.07e-5 (0–4.28e-3)	2.60	1.16e-6	1.14e-6
Tolerance concentration (mg/L)	1.00 (0.571–1.79)	266	13.7	4.97
Decrease of length at first reproduction because of chemical stress [−]	0.192 (0.174–0.210)	0.570	3.32	0.947
Decrease of oxidative damage because of chemical stress [−]	NA	NA	0.464	3.15
Increase of oxidative damage related to costs for growth [−]	35.6 (31.8–39.9)	NA	NA	NA

[a] NA = not applicable; NE = not estimated; [−] = dimensionless.
[b] Ranges in parentheses are the 95% likelihood-based confidence intervals.

is a one-compartment toxicokinetic model. The internal concentration affects the probability of dying as well as a parameter of the animal model (e.g., the maintenance costs or the assimilation of energy from food). The animal model is based on the DEB theory [6]. The DEBtox approach has been adapted to deal with life-cycle toxicity studies and the specific details of the nematode life cycle [8]. The performance of this model for nematodes has been demonstrated previously for the effects of cadmium on *C. elegans* [11], and the same approach is followed in the present study to analyze the data sets for cadmium, carbendazim, and pentachlorobenzene on *A. nanus*.

As with sexually reproducing *C. elegans* [11], *A. nanus* apparently can reduce its size at first reproduction when exposed to toxicants. An extra parameter is included to describe this behavior, although a more in-depth investigation is needed on the consequences for resource allocation.

The model was implemented in Matlab® Version 7.0 (Release 14; The MathWorks, Natick, MA, USA). The model was fitted to the data for survival, growth, and reproduction simultaneously using maximum likelihood estimation, resulting in a single set of parameter estimates for each compound. The model fits suggest the toxic mode of action of the compound, based on resource allocation, and estimates for parameters governing toxicokinetics, toxicity, and basic physiology (Table 1). Confidence intervals were generated using profile likelihoods (see Meeker and Escobar [16]). The intrinsic rate of population increase was calculated according to the Euler–Lotka equation as a function of exposure concentration (see Jager et al. [8]). This population growth rate is calculated both using the model predictions as well as directly from the effects data themselves. For the latter, survival and reproduction are interpolated on a smaller time grid (using piecewise cubic Hermite interpolation in MatLab) and subsequently integrated in the Euler–Lotka equation. Simulations were made for the population growth rates under different temperatures. These predictions can be made by assuming that temperature affects all rate constants according to the Arrhenius relationship (see Jager et al. [10]) and that the intrinsic sensitivity of the organisms is not affected by temperature. Although the first assumption appears to be quite reasonable for *A. nanus* [10], the latter assumption is more questionable, because it seems that temperature can affect the intrinsic sensitivity [17].

RESULTS

Model fits were obtained for the different life-history traits of *A. nanus* in exposure to carbendazim (Fig. 2), cadmium (Fig. 3), and pentachlorobenzene (Fig. 4). Each scenario represents the toxic effects on survival, reproduction, and growth throughout the life span of the organism.

In the case of carbendazim, the top of Figure 2 presents the model fits for the first experiment, in which the individuals were exposed to the toxicant since the egg stage at concentrations ranging from 0.15 to 0.75 mg/L agar. Even low concentrations of this compound severely affected egg hatching in *A. nanus*, making it difficult to obtain enough individuals for the experiment. Therefore, exposure to carbendazim since the egg stage could be performed only at low concentrations, giving, as a result, very low sublethal effects on the observed life-history traits. The model fits showed very small reductions in survival, reproduction, and growth with increasing concentration of the compound (Fig. 2, top). To obtain more visible sublethal effects, the individuals were hatched in control conditions and then transferred to higher concentrations, ranging from 0.3 to 1.5 mg/L agar (Fig. 2, bottom). In this case, the survival curves presented a small reduction in the lower concentrations, which then gave way to a sharp decrease in the

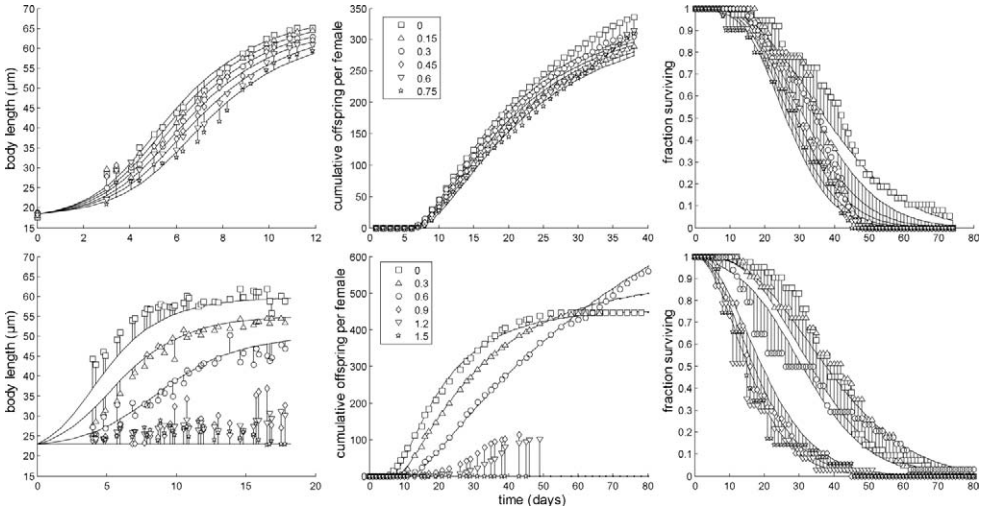

Fig. 2. Data and simultaneous model fits for all endpoints of the life-cycle study with *Acrobeloides nanus* exposed to carbendazim. These endpoints include growth, cumulative reproduction, and survival. The first experiment (top) shows individuals hatched in carbendazim. The second experiment (bottom) shows individuals hatched in control conditions. Lines connect the data points to the corresponding modeled concentration. Groups of stacked data points indicate no changes of the parameter in that time range.

three highest exposures. The reproduction curves show a substantial decrease, especially at early and intermediate time points, but exhibits an apparent recovery toward the end of the reproductive period, when the exposed individuals reach a cumulative reproduction that even exceeds that observed in control conditions. Finally, the growth curves presented a clear reduction with increasing concentrations that was maintained over the entire duration of the experiment. In the three highest concentrations, substantial mortality occurred, and the remaining nematodes hardly grew at all. Nevertheless, some of these individuals were still able to reproduce to some extent. This behavior could not be covered by the same set of model parameters used to describe the other concentrations, and we decided to leave the three highest concentrations out of the model fits for growth and reproduction.

In the cadmium scenario (Fig. 3), the survival curves of the two lowest concentrations presented a large reduction with respect to the control, and higher concentrations from this point on also caused a reduction, although to a lesser degree. Reproduction presented a gradient of effects similar to that of survival, with the largest decrease observed at the two lowest concentrations. The growth curves in this case decreased gradually with increasing concentrations of cadmium in the early and intermediate time points, and they exhibited an apparent recovery toward the end that reduced the differences in maximum size between the concentrations.

The last scenario shows the effects of pentachlorobenzene (Fig. 4). In this case, the survival curves presented a gradual decrease with increasing concentrations. Survival under control conditions deviated from the expected behavior, showing higher survival between approximately 20 to 50 d than observed in the previous experiments. Reproduction and survival also presented a gradual decrease with increasing concentrations of this compound.

The model fits show how the life-history traits present different response patterns to each of the selected toxicants. The response in each scenario is described by the model based on physiologically relevant parameters (Table 1) and assuming a mode of action that provides the best fit for the data (Table 2). For carbendazim and cadmium, a single mode of action was not sufficient to describe the time course of reproduction during the latter part of the experiment. The reproduction rate decreases with time because of aging, presumably as a result of the detrimental accumulation of oxidative damage (see Alda Álvarez et al. [11]). We therefore attribute the deviating behavior for these compounds to an interaction with the production of reactive oxygen species (ROS), although this remains speculative. In the case of carbendazim, the best model fits were obtained when the toxic mode of action was assumed to be a decrease in assimilation (see DEB scheme in Fig. 1) with an additional decrease in ROS production. For cadmium, the predicted mechanism was an increase in costs of growth with an additional increase in ROS production, whereas for pentachlorobenzene, the fits revealed a decrease in assimilation as the mode of action of the compound.

The life-history data for each of the three compounds were

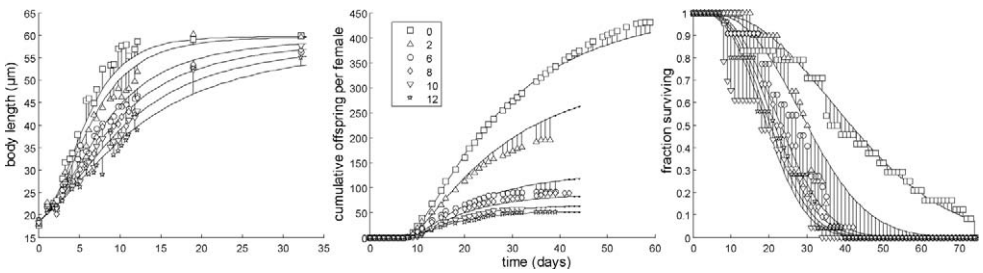

Fig. 3. Data and simultaneous model fits for growth, cumulative reproduction, and survival from the life-cycle study with *Acrobeloides nanus* exposed to cadmium. Lines connect the data points to the corresponding modeled concentration.

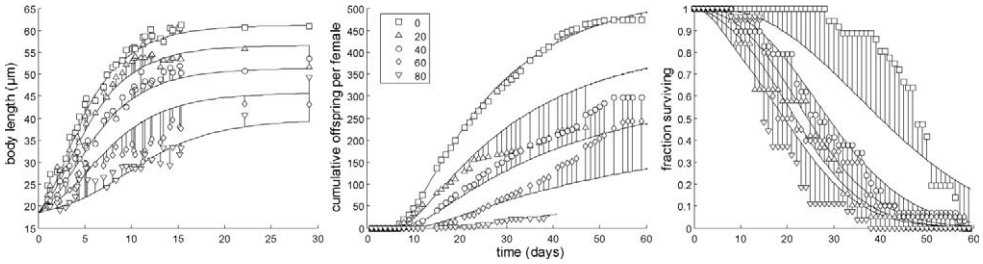

Fig. 4. Data and simultaneous model fits for all endpoints (growth, cumulative reproduction, and survival) of the life-cycle study with *Acrobeloides nanus* exposed to pentachlorobenzene. Lines connect the data points to the corresponding modeled concentration.

used to calculate the effects on the population growth rates for the different toxic concentrations (Fig. 5). The different shapes of the population growth rate curves for each compound reflect how each mode of action affects the population growth rate in a different way. The DEBtox model also predicts the effect of different temperatures (a common situation in the field) on the population growth rates for the different concentrations of toxicants. The modeled temperatures (25, 20, and 15°C) give way to a visible reduction in the population growth rates as the temperature decreases, which in combination with the increasing concentrations of toxicants leads to different patterns of effects.

DISCUSSION

Modes of action

Carbendazim. Two data sets were obtained for the carbendazim experiments (Fig. 2): One for individuals that actually hatched on carbendazim, and one for those hatched on uncontaminated agar and were subsequently transferred to contaminated medium. These two data sets were fitted separately, because they clearly differed in several parameters (see Table 1). The effect of carbendazim on hatching was not investigated further, although it is certainly relevant ecologically. For a proper DEBtox-related analysis, it first should be elucidated whether the developing embryos are affected through a maternal effect or after leaving the mother via uptake of the compound through the egg wall.

Most interestingly, the worms that hatched under uncontaminated conditions turned out to be more sensitive (a lower tolerance concentration) than the ones that successfully hatched under conditions of carbendazim exposure. At the three highest concentrations, the individuals even failed to grow and hardly reproduced (these concentrations were not included in the fit). It is unclear whether this difference resulted from the experimental procedure (i.e., hatching with or without toxicant, causing selection of the least sensitive individuals or adaptation to the toxicant) or simply from interexperimental variation. Additionally, no difference in sensitivity was observed regarding mortality in the two experiments.

For this compound, it was difficult to identify an adequate mode of action. Both a decrease in assimilation and an increase in maintenance costs can be used to fit the data. We chose effects on assimilation, because this yielded a slightly better fit (although further in-depth experimental work is needed to settle this matter). This mode of action provides a good fit to the growth data as well as the initial part of the reproduction curve. However, at later time steps, a peculiar pattern was observed in the second data set: Animals exposed to carbendazim seemed to age more slowly than expected. At the third concentration, aging was almost completely stopped (Fig. 2, bottom), as judged from the straight line for the cumulative reproduction, even though exposure to the chemical causes mortality. A decrease in aging related to a decrease in growth is expected (see Jager et al. [8]), but this effect on aging is larger than predicted. We implemented this behavior by assuming that stress on assimilation is linked to a decrease in ROS production.

The relation between ROS production and aging has been studied extensively since the development of the free radical/oxidative stress theory of aging [18], in which aerobic respiration produces free radicals that cause damage to cell components. This damage accumulates in the cell, leading to the aging phenotype. In the case of carbendazim, the mode of action indicates a decrease in ROS production and, thus, a slower pattern of aging. Although this explanation for the effects on aging that we observed with carbendazim remains speculative, it offers insight regarding further experimental testing to unravel the mechanisms at the molecular level.

Cadmium. Figure 3 shows that the model provides a good fit to the data. The growth pattern clearly reflects the mode of action ''costs of growth,'' presenting slower growth at increasing concentrations but reaching a final body size similar to that of the controls. The rationale behind this mode of action is that the compound increases the energetic costs of making new tissue. This slows down the rate of growth, but not the ultimate size, which is reached when all the assimilated resources are used for maintenance (neither assimilation nor maintenance costs per unit of body size are related to the costs

Table 2. Predicted physiological mode of action of the selected compounds on *Acrobeloides nanus* and *Caenorhabditis elegans*

Compound	*Acrobeloides nanus*	*Caenorhabditis elegans*[a]
Carbendazim	Decrease in assimilation with an additional effect on reactive oxygen species (ROS) production (slower aging)	Decrease in assimilation
Cadmium	Increase in costs for growth with an additional effect on ROS production (faster aging)	Decrease in assimilation
Pentachlorobenzene	Decrease in assimilation	Increase in costs of growth and costs for reproduction with no toxic effects on survival

[a] *Caenorhabditis elegans* modes of action are based on previous studies from Alda Álvarez et al. [11,12].

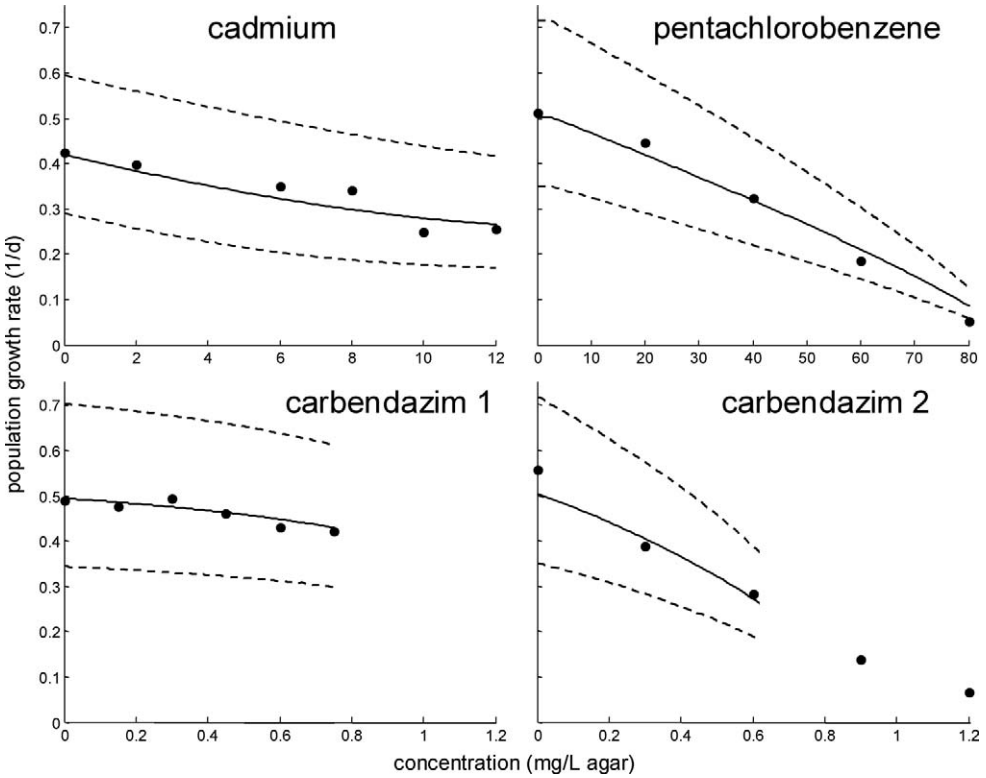

Fig. 5. Modeled intrinsic rate of population increase for *Acrobeloides nanus* exposed to the three compounds. Carbendazim 1 corresponds to the experiment in which individuals were hatched in carbendazim, whereas Carbendazim 2 corresponds to individuals hatched under control conditions. Solid circles represent population growth rates calculated directly from the life-history data (at 20°C). Lines represent the DEBtox fits for the test temperature (20°C; solid line) and predictions for other temperatures (25°C [top] and 15°C [bottom]; broken line).

of growth). The apparent recovery of the body size in Figure 3 therefore is simply a consequence of this mode of action and is not related to a reversal of toxicity.

The effect of cadmium on body size has immediate repercussions for reproduction, which are reflected as a delay in the start of reproduction with increasing concentrations (although this effect is not very large, because the toxic stress at the same time decreases the size at first reproduction; see *Materials and Methods*). Reproduction also starts at a slower rate, because body size determines egg production. Nevertheless, this is not sufficient to explain the observed pattern of egg production over time. It appears that in the cadmium-exposed individuals, the rate of reproduction slows down more rapidly than expected. An adequate description of this pattern can be provided by assuming that cadmium stress also increases the ROS production related to growth, leading to a faster pattern of aging. Previous studies have suggested that cadmium treatment causes lipid peroxidation [19], with production as a consequene of ROS [20]. Alternatively, it can replace iron from cellular binding sites to increase the free iron level and lead to iron-induced oxidations (Fenton-type reaction) [21] or increase ROS by reducing the cellular antioxidative capacity [22]. Indeed, an increase in hydrogen peroxide, superoxide anion, and hydroxyl radicals after cadmium exposure has been reported in several studies [23–25].

Pentachlorobenzene. Figure 4 shows that the model provides good fits for the growth data, but reproduction seems to be more variable in the exposed individuals (although these deviations occur when most of the animals have already died). Survival data show a less satisfactory fit with a control pattern that is very different from that observed in the other two compounds; however, the reason for these deviations remains unclear.

The mode of action ''assimilation'' suggests that exposure to pentachlorobenzene apparently decreases the energy intake into the organism (which also holds for carbendazim, as mentioned previously). This can be achieved either by decreasing the feeding rate or by decreasing the efficiency with which the food is assimilated into the reserves [7]. The last assumption is difficult to test, but previous experiments with the nematode *C. elegans* have shown feeding inhibition during exposure to sublethal concentrations of cadmium [26], which suggests a decrease in assimilation when modeled with DEBtox [11], thus supporting the first assumption as a possible explanation. This mode of action is characterized by a decreased growth rate and a lower ultimate body size. As stated earlier, growth stops when all the assimilated energy is used for maintenance. A lower assimilation rate thus restricts the body size that can be achieved.

Population-level effects

The intrinsic rate of population increase integrates the time course of effects on all endpoints into an ecologically relevant parameter [9] that bears a direct relation with the protection goals of environmental risk assessment (populations and ecosystems). Toxic compounds will exert their effect at different levels, but essentially, we are interested in how these effects are reflected by populations. The dissection of the mode of action of different compounds allows extrapolation into population-level effects. Depending on the physiological mode of action of the compound, the effects on the population growth rates differ (Fig. 5). At this level, the effects of ROS production

(and its interaction with the toxicant) have little relevance, because the early offspring contribute most to the population growth rate. The estimated population growth rate in Figure 5 is based on the model fits in Figures 2 through 4, so this parameter also can be calculated for untested concentrations (hence the plotted line). Furthermore, the growth rate is calculated directly from the data (without any model whatsoever; see *Materials and Methods*), which can be done only for the tested concentrations (and, hence, the individual symbols).

In the case of cadmium, an increase in the costs of growth as the concentration increases leads to a gradual decrease of the population growth rate that seems to slow down at the higher concentrations. On the other hand, the decrease in assimilation caused by pentachlorobenzene and carbendazim (experiment 2) leads to a faster decrease of the population growth rates that tends to become more severe at the higher concentrations. In the first carbendazim experiment, in which the individuals were hatched in exposure to the toxicant, there seems to be a less sensitive response to the compound compared with that observed in the second experiment. A possible explanation is that either hatching in carbendazim selects the less sensitive individuals or causes a change in the pathways of energy metabolism. Note that the highest concentrations were not used for the model fit in Figure 2; therefore, the estimated population growth rate does not extend beyond 0.6 mg/L agar. It also should be noted that the unquantified effect of carbendazim on the hatching success was not included in the estimate of the population growth rate in Figure 5. Thus, in practice, the effects of carbendazim will be worse than are shown in this figure.

When temperature effects are incorporated into the simulations, a substantial decrease in the population growth rates as temperature decreases is visible in the four scenarios. However, the interactions between temperature and toxicity differ in each case. With cadmium, the differences between the population growth rates at each temperature are maintained throughout the different concentrations of toxicant. However, with pentachlorobenzene and carbendazim, the effects of temperature are smaller with increasing concentrations, as can be observed from the proximity of the curves (this is visible most clearly in the second carbendazim experiment). Such simulations are of interest, because different temperature regimes that can be found in field conditions can severely affect the way that populations respond to a toxic stress.

Interactions with life-history characteristics

Previous studies using DEBtox have been carried out with another nematode species, *C. elegans* [11,12], which has a different life-history strategy than *A. nanus*, a parthenogenetic species in which the ovum develops into a new individual without fertilization. The reproductive period lasts approximately 40 d (at 20°C), during which they produce a total of 400 to 500 progeny [10]. The average life span is from 50 to 60 d, placing this organism in the category of intermediate-life-span nematodes. On the other hand, *C. elegans* is a facultative, self-fertilizing hermaphrodite that also can reproduce sexually in the presence of males. Males occur at very low frequencies in natural populations [27], and hermaphroditic reproduction therefore is the most common. This mode of reproduction implies that one individual produces both male and female gametes to self-fertilize. In *C. elegans*, this yields a total of approximately 300 progeny in a reproductive period that lasts 5.5 d (15°C) [11]. These nematodes present short life spans of approximately 20 d at 15°C.

In *C. elegans*, cadmium was reported to act through a decrease in assimilation [11], as was the case for carbendazim, and pentachlorobenzene presented a combination of costs of growth and costs of reproduction, with no toxic effects on survival [12] (Table 2). These modes of action therefore are different from those that we find in *A. nanus* except for the case of carbendazim, which acts through a decrease in assimilation in both species (although showing an extra effect on ROS production in *A. nanus*). This indicates that the life-history characteristics of each organism have a clear influence on a chemical's physiological mode of action, which has important consequences for structure-based classifications of chemical modes of action that do not consider these interactions. For example, pentachlorobenzene generally is classified as a narcotic but appears to exert its toxic effects on energy allocation through different mechanisms depending on the life-history strategy of the organism. This difference in mode of action may reflect physiological differences between the species. For instance, some of the differences in the effects on reproduction and the additional ROS effect on it may relate to the difference in reproductive strategy between the two nematode species.

Our simulations suggest that different physiological modes of action affect population growth rates in different ways. It therefore is of great importance to understand the details of the mechanisms through which a compound exerts toxicity, because this will allow more accurate predictions of the population-level effects from different compounds and, therefore, will provide an important tool for risk assessment. Risk assessment approaches that rely on a strict chemical-class perspective for the analysis of toxic stressors could benefit from evolving toward the incorporation of methodologies that are more consistent with actual resulting mechanisms of action.

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