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Interactions between toxic chemicals and natural environmental factors — A meta-analysis and case studies

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ABSTRACT

The paper addresses problems arising from effects of natural environmental factors on toxicity of pollutants to organisms. Most studies on interactions between toxicants and natural factors, including those completed in the EU project NoMiracle (Novel Methods for Integrated Risk Assessment of Cumulative Stressors in Europe) described herein, showed that effects of toxic chemicals on organisms can differ vastly depending purely on external conditions. We compiled data from 61 studies on effects of temperature, moisture and dissolved oxygen on toxicity of a range of chemicals representing pesticides, polycyclic aromatic hydrocarbons, plant protection products of bacterial origin and trace metals. In 62.3% cases significant interactions ($p \leq 0.05$ or less) between natural factors and chemicals were found, reaching 100% for the effect of dissolved oxygen on toxicity of waterborne chemicals. The meta-analysis of the 61 studies showed that the null hypothesis assuming no interactions between toxic chemicals and natural environmental factors should be rejected at $p = 2.7 \times 10^{-82}$ (truncated product method probability). In a few cases of more complex experimental designs, also second-order interactions were found, indicating that natural factors can modify interactions among chemicals. Such data emphasize the necessity of including information on natural factors and their variation in time and across geographic regions in ecological risk assessment. This can be done only if appropriate ecotoxicological test designs are used, in which test organisms are exposed to toxicants at a range of environmental conditions. We advocate designing such tests for the second-tier ecological risk assessment procedures.

1. Introduction

It has been shown in a number of studies that natural environmental conditions can significantly modify responses of organisms to toxicants (e.g., Cooney et al., 1983; Bryant et al., 1985; Spurgeon et al., 1997; Donker et al., 1998; Sjursen and Holmstrup, 2004; Heugens et al., 2006; Khan et al., 2007). Even earlier it was known that chemicals themselves can interact significantly with each other, showing either antagonistic (less than additive effects) or synergistic (more than additive effects) behaviour (e.g., Doelman et al., 1984; Pape-Lindstrom and Lydy, 1997; Forget et al., 1999; Van der Geest et al., 2000). These two phenomena combined together may lead to serious deviations of actual toxicity from that predicted by standard ecotoxicological tests because all current standard tests are performed with single toxicants under some "standard" conditions. These "standard conditions" usually mean that animals are kept at a constant and optimal temperature and moisture, pH or dissolved oxygen levels, being either fed *ad libitum* or starved. The examples include Daphnia immobilization test (OECD 202) in which daphnids are exposed to a toxicant for 48 h at 18–22 °C without feeding; fish acute toxicity test (OECD 203) with fish kept for 96 h in water with near-saturated oxygen concentrations (≥90%), at a temperature optimal for the test species (varying ±2 °C) and with no food; earthworm reproduction test (OECD 222) which should be run on worms fed with manure for 8 weeks at 20±2 °C and 40–60% of water holding capacity (WHC). These are certainly not the conditions that an animal is usually exposed to in the field, where large fluctuations in climatic factors as well as in food availability are the norm. Moreover, because of large climatic differences between different regions of Europe (and even larger for the whole world), results of such standard tests can be representative for only a narrow strip of Earth where average climatic conditions resemble those used in the particular ecotoxicological tests.

Life in the real world is much more complicated than standard ecotoxicological tests can handle and in this article we point out the

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fact that this mismatch between laboratory and field conditions can lead to errors in ecological risk assessment. Using mostly data generated under the EU NoMiracle project (Novel Methods for Integrated Risk Assessment of Cumulative Stressors in Europe; http://www:nomiracle.jrc.it), but including also studies published earlier by NoMiracle participants, we show how common the interactions between natural factors and pollutants are, and propose to incorporate, at least partly, the real-world complexity into ecotoxicological tests and ecological risk assessment. Because specific problems of toxicity of mixtures of chemicals, including different shapes of the three-dimensional dose–response surface describing interactions among toxicants, were discussed extensively before (Jonker et al., 2005; Barata et al., 2006; Loureiro et al., 2009; Martin et al., 2009), we concentrate herein on consequences of interactions between pollutants and natural environmental factors. In fact, under certain circumstances, this problem may be even more important than interactions among chemicals because, as noted by some researchers, either similar action (SA) or independent action (IA) models can offer sufficiently good approximation of mixtures toxicity (e.g., Faust et al., 2003). If this is true, then it may appear that combined effects of suboptimal natural environmental conditions and toxic chemicals are more important than interactions among chemicals. The knowledge about these interactions (Parker et al., 1999) and how they affect organisms' fitness is surprisingly scarce.

Although only in recent years this topic gained a major interest among scientists, the issue is actually not that new. For example, already over 20 years ago Bryant et al. (1985) noticed that salinity and temperature may significantly affect results of aquatic toxicity tests. This was especially clear for *Macoma baltica*, in which the median survival time at the very same concentration of nickel could be as low as 50 h or as long as ca. 300 h — a 6-fold difference, depending solely on the combination of water salinity and temperature. In the very same year Demon and Eijsackers (1985) published a paper on effects of extreme temperatures, temperature fluctuations and moisture on the toxicity of two pesticides to two species of soil invertebrates: an isopod and a springtail. It appeared that the combined effect of the pesticides and high temperature was multiplicative, and the springtails were more susceptible to pesticides at desiccating conditions. Consequently, they concluded that unfavorable environmental conditions must be incorporated into assessment procedures. In the last 10 years, the number of papers pointing on such interactions increased substantially, showing also effects of other natural stressors on toxicity of a range of chemicals. For example, it was shown how drought and frost can interact with copper toxicity to earthworms (Holmstrup et al., 1998), that the ability of springtails to tolerate drought is impaired by copper exposure (Holmstrup, 1997) or that exposure to pyrene increases springtail's sensitivity to cold and drought stress (Sjursen and Holmstrup, 2004). Similarly, previous exposure to polycyclic aromatic hydrocarbons (pyrene and fluorene) and a detergent (nonylphenol) increased the susceptibility of the collembolan *Folsomia candida* to drought stress (Sørensen and Holmstrup, 2005). In the earthworm *Dendrobaena octaedra* "synergistic interactions" (i.e., the observed effect was higher than at optimal temperature; see below) between nonylphenol and high temperatures were observed by Jensen et al. (2009). High temperature also increased the toxicity of chlorpyriphos in the moths *Earias vitella* (Satpute et al., 2007). These examples show that interactions between chemicals and natural factors may be complex in their nature, modifying toxic effects either through direct effects on organisms or by affecting chemical/biochemical pathways of the toxicants themselves.

Studies involving three-factor interactions are much scarcer. Nevertheless, the existing ones indicate possible significant higher-order interactions, like in the study by Heugens et al. (2006) on *Daphnia magna*, which revealed that the influence of food availability on cadmium toxicity was stronger at high temperatures. Also Bednarska et al. (2009) showed a significant effect of temperature on the interaction between nickel and chlorpyriphos toxicity to the ground beetle *Pterostichus oblongopunctatus*.

Suboptimal environmental conditions may result in more serious effects of pollutants than observed under optimal circumstances but opposite effects have also been observed. There is thus some similarity to what is called synergism and antagonism in interactions between toxic chemicals. However, in this case the terms have to be used cautiously, because in contrast to interactions between toxicants no clear baseline model, such as SA or IA, can be formulated for interactions between chemicals and natural factors. We recognize that these models are used sometimes successfully to describe also interactions between chemicals and natural factors but we stress that this results from purely phenomenological similarity to interactions among chemicals and none of these models relates to actual physiological and biochemical processes that stand behind interactions between natural factors and toxicants. Whenever we thus use these terms for describing effects of natural factors on toxic effects of chemicals, they should be understood as purely phenomenological description of effects higher ("synergism") or lower ("antagonism") than would be expected under optimal environmental conditions.

In some papers authors relate to certain environmental conditions as "natural stressors" or "natural stressing factors". Although we admit that in a number of circumstances such a nomenclature is fully justified as indeed natural factors can exert significant stress on organisms, we avoid using such terms in this work because it is frequently difficult to delineate strictly "optimal conditions" from "suboptimal" from "stressful". We thus prefer to talk more generally about interactions between *natural factors* and toxicants. Such an approach relieved us from arbitrary selecting for the meta-analysis those studies that could be strictly classified as examples of effects of "natural stressors" as we believe that such a distinction is impossible to make. Moreover, from the point of view of ecological risk assessment, more general information about effects of natural environmental factors, not necessarily at highly stressing levels, is more interesting and useful.

In the NoMiracle project the interactions between toxic chemicals and natural factors were studied on a broad range of species, ranging from potworms to vertebrates. In many of these studies some interactions between chemicals and natural factors were found, strengthening the assumption about probable importance of suboptimal temperatures, drought, etc., for ultimate effects of toxicants on organisms. The main aim of this article is thus to summarize these findings and to perform a meta-analysis of the data available in the NoMiracle consortium on interactions between toxicants and natural environmental conditions.

Probably the most important natural factor, which is highly variable in the field and is of major importance for the physiological state of an organism, is temperature. Those ecotoxicological studies in which effects of temperature were investigated in terrestrial (e.g., Abdel-Lateif et al., 1998; Sjursen and Holmstrup, 2004; Bindesbøl et al., 2005; Bednarska and Laskowski, 2008) and aquatic invertebrates (e.g., Heugens et al., 2003), confirm its importance for effects of pollutants. Proper information on toxicity of chemicals under different temperature regimes may lead to better extrapolation of results from standard ecotoxicological assays to field conditions. For this reason, in the second part of the manuscript we focused on temperature effects on toxicity of chemicals and we provide two examples (case studies) on effects of toxicants under different temperatures. The first example illustrates an attempt to extrapolate laboratory-derived data on combined effects of temperature and phenanthrene on potworms to predict ecotoxicological risk across Europe. In the second one we use data from a published work (Bednarska et al., 2009) to show that temperature not only can modify effects of chemicals directly but also through influencing interactions among toxicants.

2. Methods

Due to the nature of the article, which summarizes results from a number of studies on effects of natural factors on toxicity of pollutants to a range of organisms, we do not describe details of each study here. For such details, the reader should refer to original papers or consult the authors in case of not published studies. Although coordinated across the NoMiracle project as far as possible, all the studies on which this article is based used somewhat different experimental designs and different environmental conditions, as required for specific test organisms and their environments. Also, although all data have been obtained directly from the NoMiracle project participants, we also use older data, generated under other research projects. All studies on interactions between toxic chemicals and natural factors, which have been collected directly from NoMiracle participants, are summarized briefly in Table 1. Only these data were utilized in meta-analysis in order to avoid any bias towards significant (or nonsignificant) results.

The meta-analysis was done first by summarizing significant *vs.* nonsignificant interactions, disregarding information on the type of chemicals and natural factors as well as the shape of interactions ("synergistic" *vs.* "antagonistic"). All studies were treated equally in terms of their validity as all were collected directly from the researchers. They were also performed mostly using similar standards and experimental designs. In most studies several endpoints were measured (up to 24 per single test) and, thus, a number of different interactions were possible for a single study. To avoid pseudoreplication, we treated each study as an independent replicate and counted the study only once as indicating significant interaction even if several interactions have been found significant. Similarly, each study in which no significant interactions were found was counted only once, even if different endpoints were measured and several interactions were tested. This allowed us to calculate the frequency of studies with significant interactions between chemicals and natural factors. Then, the truncated product method (TPM) described by Zaykin et al. (2002) was used to determine whether indeed the deviation from the non-interaction null hypothesis was significant across all 61 studies. Briefly, the method relies on calculating the product of all significant p values (in this case we used $p \leq 0.05$) in a set of H_0 hypothesis tests and calculating the probability of such a product or a smaller value under the overall hypothesis that all H_0 hypotheses are true (TPM-p value; Zaykin et al., 2002). The probability was estimated with the TPM program available at ftp://statgen.ncsu.edu/pub/zaykin/tpm. The significance of the TPM indicates that at least one false null hypothesis can be found among the studies in which $p \leq 0.05$ for interactions between chemical and natural factors were found. In cases of multiple endpoints and interactions per study (see above), we used only one, lowest p value reported in that study. Because with such a procedure a single highly significant result (an extremely low p value) could bias the final outcome seriously and, additionally, some publications reported only whether interactions were significant ($p \leq 0.05$) or not, we adopted also a more conservative approach by assigning the value 0.05 to all significant interactions.

After rejecting the overall H_0 hypothesis, we tested the interactions for false discovery rate (FDR) with the method proposed by Benjamini and Hochberg (2000). In this procedure, for n tests the FDR is controlled at the desired level α by first ranking all p values in ascending order ($p_1 \leq p_{2n} \leq \ldots \leq p_n$) and then checking if $p_i \leq (\alpha/n)i$, where i is the rank. For all tests fulfilling the criterion, null hypotheses are rejected.

The illustrative part of the manuscript, aiming at showing how ecotoxicological risk of chemicals can change with temperature, was built on two examples. For the first one, from among the studies presented in Table 1, one case study was selected showing significant interactions between temperature and phenanthrene in the potworm *Enchytraeus doerjesi*. The relationship estimated from the experimental data (the response surface equation) was applied across Europe in order to predict potential temperature-specific effects of phenanthrene at two different pollution scenarios: 200 and 800 mg kg^{-1} soil. The scenarios are presented for the beginning of the growing season, using average maximum April temperatures. Predicted effects of phenanthrene at different climatic conditions are presented as maps of the estimated population growth rate λ. Although this does not translate yet directly to actual ecological risk, this exercise shows how large the differences in predicted effects of a toxicant at specific concentration can be, depending solely on temperature variation. In the second example we used the data on joint effects of chlorpyriphos, nickel and temperature on the ground beetle *P. oblongopunctatus* (Bednarska et al., 2009). This study was chosen because it is one of the very few existing studies on higher level interactions between chemicals and temperature and the one for which we had raw data. The data were used to estimate the beetles' life time under different combinations of exposure to chemicals at a range of temperatures. This allowed us to show that temperature interacts not only directly with chemicals but also affects interactions between chemicals. For clarity, specific methods used in both case studies are given directly with the studies.

3. Results and discussion

Altogether results from studies on 61 combinations of different ectothermic animals and stressors have been provided by the NoMiracle project participants. Some studies on specific interactions in a single species employed more than one experiment (e.g., different endpoints required different test lengths — see Table 1) but for the purpose of meta-analysis they were treated as one study as long as they were performed by the same research group as part of one research project. This was the specific combination of test species, tested chemical and natural stressor(s) that defined a study. This let us be sure that all studies can be treated as independent. Among such delineated studies, 51 were done on invertebrates, and 10 on vertebrates, the latter represented by the single fish species *Danio rerio*. Invertebrates covered cladocerans (5 studies on *D. magna*), potworms (8 studies on *E. doerjesi*), earthworms (17 studies: 3 on *Aporrectodea caliginosa*, 12 on *D. octaedra*, 2 on *Lumbricus rubellus*), springtails (19 studies: 17 on *F. candida* and 2 on *Protaphorura fimata*), and ground beetles (2 studies on *P. oblongopunctatus*). The animals represented both aquatic (*D. magna*, *D. rerio*) and terrestrial (all remaining species) organisms. The chemicals used in tests covered a broad range of organic and inorganic pollutants, and temperature, air humidity (relative humidity, RH), soil moisture (% maximum water holding capacity, WHC) and oxygen saturation in water were used as natural environmental factors.

The studies showed that interactions between environmental factors and toxicants is a common phenomenon and can take complicated shapes. Detailed list of all studies used in this article is provided in Table 1. Among the 61 studies significant interactions were found in 38 cases, which represent 62.3%. Temperature affected toxicity in 62.1% studies (18 cases out of 29), and significant interaction between soil moisture or air humidity and toxic chemicals was found in 13 cases out of 25 (52%). For freshwater organisms, dissolved oxygen affected toxicity of chemicals in all of seven cases. This so well pronounced interaction between toxic chemicals and dissolved oxygen is particularly worth underlining as, in turn, oxygen solubility in water depends on temperature. A possibility of such a complicated interaction between temperature, oxygen concentration and toxicity of chemicals was pointed already years ago by Alabaster and Welcomme (1962). This indicates also on a possibility of second-level interactions.

Indeed, second-order interactions were also found in this study, indicating that natural factors can modify interactions among chemicals. Among the studies collected in Table 1, only two tested higher-order interactions and both revealed significant second-order

Table 1
Summary of the studies provided by NoMiracle participants on interactions between toxic chemicals (toxicant range: concentrations in mg kg^{-1}, or mg l^{-1}, as appropriate) and natural factors (ranges for: temperature in °C, soil moisture in % water holding capacity, air humidity in % relative humidity, and dissolved oxygen in mg l^{-1}). For the studies where the interactions were tested against specific model (similar action, SA, or independent action, IA), the table reports against which model a significant interaction was found; for the studies with more than two factors, all significant interactions are specified. Studies indicating significant interactions after controlling for false discovery rate (Benjamini and Hochberg, 2000) are marked with asterisks next to the study number (No.).

No.	Organism	Toxicant (range)	Natural factor (range)	Test duration (exposure route)	Endpoint (analysis method)[a]	Interactions[b]	Reference
1*	*Enchytraeus doerjesi*	Abamectin (0–104)	Temperature (10–30)	4 weeks (sand)	Final population number (GLM) Population growth rate (GLM)	$p<0.0001$ $p<0.0001$	Kramarz, unpublished[d]
2*	*Enchytraeus doerjesi*	Phenantrene (0–800)	Temperature (10–30)	4 weeks (sand)	Final population number (GLM) Population growth rate (GLM)	$p<0.0001$ $p=0.0005$	Kramarz, unpublished
3*	*Enchytraeus doerjesi*	Chlorpyriphos (0–500)	Temperature (10–30)	4 weeks (sand)	Final population number (GLM) Population growth rate (GLM)	$p=0.0008$ $p=0.0003$	Kramarz, unpublished
4	*Enchytraeus doerjesi*	Nickel (0–4)	Temperature (10–30)	4 weeks (sand)	Final population number (GLM) Population growth rate (GLM)	None None	Kramarz, unpublished
5	*Enchytraeus doerjesi*	Abamectin (0–104)	Moisture (10–70)	4 weeks (sand)	Final population number (GLM) Population growth rate (GLM)	None None	Kramarz, unpublished
6*	*Enchytraeus doerjesi*	Chlorpyriphos (0–500)	Moisture (10–70)	4 weeks (sand)	Final population number (GLM) Population growth rate (GLM)	$p<0.0001$ $p<0.0001$	Kramarz, unpublished
7	*Enchytraeus doerjesi*	Nickel (0–4)	Moisture (10–70)	4 weeks (sand)	Final population number (GLM) Population growth rate (GLM)	None None	Kramarz, unpublished
8	*Enchytraeus doerjesi*	Copper (0–8)	Moisture (10–70)	4 weeks (sand)	Final population number (GLM) Population growth rate (GLM)	None None	Kramarz, unpublished
9	*Aporrectodea caliginosa*	Copper (0–20)	Air relative humidity (97.5–100)	14 days exposure in water followed by 14 days exposure to drought	Survival of egg capsules (hatchability) ANOVA	$p<0.05$	Holmstrup et al., 1998
10	*Aporrectodea caliginosa*	Copper (0–12)	Low temperature (−8 to 0)	14 days exposure in water followed by 28 days exposure to sub-zero temperature	Survival of egg capsules (hatchability) ANOVA	$p<0.05$	Holmstrup et al., 1998
11	*Aporrectodea caliginosa*	Copper (0–300)	Soil moisture (pF 2–4.5)	21 days (soil)	Survival Two-factor dose–response model with interaction term	$p=0.006$	Friis et al., 2004
12	*Dendrobaena octaedra*	Copper (0–8)	Low temperature (−8 to 0)	14 days exposure in water followed by 28 days exposure to sub-zero temperature	Survival of egg capsules (hatchability) ANOVA	$p<0.05$	Holmstrup et al., 1998
13*	*Dendrobaena octaedra*	Copper (0–200)	Low temperature (−8 to 2)	28 days exposure in soil, followed by 10 days exposure to sub-zero temperature	Survival Two-factor dose–Response model with interaction term	$p=0.0004$	Bindesbøl et al., 2005
14	*Dendrobaena octaedra*	Copper (0–20)	Air relative humidity (97.5–100)	14 days exposure in water followed by 14 days exposure to drought	Survival of egg capsules (hatchability) ANOVA	$p<0.05$	Holmstrup et al., 1998
15	*Dendrobaena octaedra*	Nonylphenol (0–900)	Low temperature (−6 to 1.4)	28 days exposure in soil, followed by 10 days exposure to sub-zero temperature	Survival Two-factor dose–Response model with interaction term	None	Jensen et al., 2009
16*	*Dendrobaena octaedra*	Nonylphenol (0–500)	Low temperature (25–35)	14 days exposure in soil, followed by hours heat shock	Survival Two-factor dose–Response model with interaction term	$p=0.001$	Jensen et al., 2009
17*	*Dendrobaena octaedra*	Nickel (0–250)	Low temperature (−6 to 2)	42 days exposure in soil, followed by 8 days exposure to sub-zero temperature	Survival Two-factor dose–Response model with interaction term	$p=0.02$	Bindesbøl et al., 2009
18*	*Dendrobaena octaedra*	$HgCl_2$ (0–10)	Low temperature (−6 to 2)	42 days exposure in soil, followed by 8 days exposure to sub-zero temperature	Survival Two-factor dose–Response model with interaction term	$p=0.0008$	Bindesbøl et al., 2009
19	*Dendrobaena octaedra*	Lead (0–1000)	Low temperature (−6 to 2)	42 days exposure in soil, followed by 8 days exposure to sub-zero temperature	Survival Two-factor dose–Response model with interaction term	None	Bindesbøl et al., 2009
20	*Dendrobaena octaedra*	Pyrene (0–250)	Low temperature (−6 to 2)	42 days exposure in soil, followed by 8 days exposure to sub-zero temperature	Survival Two-factor dose–Response model with interaction term	None	Bindesbøl et al., 2009
21	*Dendrobaena octaedra*	Phenanthrene (0–250)	Low temperature (−6 to 2)	42 days exposure in soil, followed by 8 days exposure to sub-zero temperature	Survival Two-factor dose–Response model with interaction term	$p=0.04$ (antagonism)	Bindesbøl et al., 2009

22	*Dendrobaena octaedra*	Abamectin (0–10)	Low temperature (−6 to 2)	42 days exposure in soil, followed by 8 days exposure to sub-zero temperature	Survival Two-factor dose–Response model with interaction term	None	Bindesbøl et al. 2009
23	*Dendrobaena octaedra*	Carbendazim (0–2.5)	Low temperature (−6 to 2)	42 days exposure in soil, followed by 8 days exposure to sub-zero temperature	Survival Two-factor dose–Response model with interaction term	None	Bindesbøl et al., 2009
24	*Lumbricus rubellus*	Fluoranthene	Soil moisture (0–15% below optimum)	28 days	Cocoon production (GLM and MixTox model)	None	Long et al., 2009
25	*Lumbricus rubellus*	Flouranthene (0–500)	Soil moisture (0–15% below optimum)	21 days exposure in soil	Survival and cocoons production Two-factor dose–response model with interaction term	None	Long et al., 2009
26	*Folsomia candida*	Copper (0–300)	Air relative humidity (96.7–100)	7 days exposure in soil, followed by 7 days exposure to drought	Survival (judged from 95% fiducial confidence limits)	$p<0.05$	Holmstrup, 1997
27	*Folsomia candida*	Nonylphenol (NP) (0–40)	Air relative humidity (96.7–100)	7 days exposure in soil, followed by 7 days exposure to drought	Survival (judged from 95% fiducial confidence limits)	$p<0.05$	Holmstrup, 1997
28	*Folsomia candida*	Linear alkylbenzene sufonate (0–300)	Air relative humidity (96.7–100)	7 days exposure in soil, followed by 7 days exposure to drought	Survival (judged from 95% fiducial confidence limits)	None	Holmstrup, 1997
29*	*Folsomia candida*	Nonylphenol (0–62.5)	Air relative humidity (97–100)	8 days exposure in soil, followed by 7 days exposure to drought	Survival Two-factor dose–Response model with interaction term	$p=0.0001$	Højer et al., 2001
30*	*Folsomia candida*	Pyrene (0–250)	Air relative humidity (98.2–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival (Kruskal–Wallis)	$p=0.013$	Sørensen and Holmstrup, 2005
31*	*Folsomia candida*	Flourene (0–160)	Air relative humidity (98.2–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival (Kruskal–Wallis)	$p=0.007$	Sørensen and Holmstrup, 2005
32*	*Folsomia candida*	Nonylphenol (0–150)	Air relative humidity (98.2–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival (Kruskal–Wallis)	$p=0.006$	Sørensen and Holmstrup, 2005
33	*Folsomia candida*	Linear alkylbenzene sulfonate (0–1000)	Air relative humidity (98.2–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival (Kruskal–Wallis)	None	Sørensen and Holmstrup, 2005
34	*Folsomia candida*	Dimethoate (0–2.5)	Air relative humidity (98.2–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival (Kruskal–Wallis)	None	Sørensen and Holmstrup, 2005
35	*Folsomia candida*	Cypermethrin (0–16)	Air relative humidity (98.2–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival (Kruskal–Wallis)	None	Sørensen and Holmstrup, 2005
36	*Folsomia candida*	Copper (0–2200)	Air relative humidity (98.2–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival (Kruskal–Wallis)	None	Sørensen and Holmstrup, 2005
37	*Folsomia candida*	Cadmium (0–1100)	Air relative humidity (98.2–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival (Kruskal–Wallis)	None	Sørensen and Holmstrup, 2005
38*	*Folsomia candida*	Nonylphenol (0–75)	Air relative humidity (97–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival Two-factor dose–response model with interaction term	$p=0.0003$	Skovlund et al., 2006
39*	*Folsomia candida*	Pyrene (0–150)	Air relative humidity (97–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival Two-factor dose–response model with interaction term	$p=0.0006$	Skovlund et al., 2006
40	*Folsomia candida*	DDE (0–300)	Air relative humidity (97–100)	21 days exposure in soil, followed by 7 days exposure to drought	Survival Two-factor dose–response model with interaction term	None	Skovlund et al., 2006
41*	*Folsomia candida*	$HgCl_2$ (0–48)	Low temperature (−7 to 0)	24 h exposure in water, followed by 2 h cold shock	Survival Two-factor dose–response model with interaction term	$p=0.0001$	Holmstrup et al., 2008
42*	*Folsomia candida*	$HgCl_2$ (0–48)	High temperature (20–35.5)	24 h exposure in water, followed by 2 h heat shock	Survival Two-factor dose–response model with interaction term	$p=0.0001$	Slotsbo et al., 2009
43	*Protaphorura fimata*	Pyrene (0–100)	Moisture (100–98.2)	21 days exposure in soil, followed by 7 days exposure to drought	Survival (ANOVA)	$p<0.05$	Sjursen and Holmstrup, 2004
44	*Protaphorura fimata*	Pyrene (0–300)	Low temperature (−3 to 20)	14 days exposure in soil, followed by 14 days exposure to low temperature	Survival (ANOVA)	$p<0.05$ (antagonism)	Sjursen and Holmstrup, 2004
45*	*Pterostichus oblongopunctatus* adults	Chlorpyriphos (CPF) (0–80) Nickel (Ni) (0–10,000)	Temperature (T) (10–25)	134 days (CPF topically; Ni with food)	Survival (GLM) Eggs per female (GLM)	Ni× CPF×T, $p=0.006$ Ni×T, $p=0.03$	Bednarska et al., 2009

(continued on next page)

Table 1 (*continued*)

No.	Organism	Toxicant (range)	Natural factor (range)	Test duration (exposure route)	Endpoint (analysis method)[a]	Interactions[b]	Reference
46*	*Pterostichus oblongopunctatus* larvae	Chlorpyriphos (CPF) (0–2) Nickel (Ni) (0–1200)	Temperature (*T*) (10–25)	125 days (CPF through soil; Ni with food)	Survival (GLM) Proportion of emerged imagines (GLM)	Ni × CPF × *T*, $p = 0.01$ CPF × *T*, $p < 0.0001$ Ni × *T*, $p = 0.03$	Bednarska and Laskowski, 2009
47*	*Daphnia magna*	Cadmium	Dissolved oxygen Acute test: 0.1–1.9 Feeding inhibition test: 2–6	48 h (in ASTM hard water)	Survival (MixTox model; χ^2 test), Survival (MixTox model; χ^2 test) Feeding rate (MixTox model; χ^2 test)	IA $p = 0.0354$ (synergism) SA $p = 1.83 \times 10^{-27}$ (antagonism) IA $p = 0.0044$ (antagonism)	Ferreira et al., 2008
				24 h exposure (in ASTM hard water)	Feeding rate (MIXTOX model; χ^2 test)	SA $p = 0.0035$ (antagonism)	
				24 h exposure + 4 h post-exposure (in ASTM hard water)	Feeding rate (MIXTOX model; χ^2 test)	SA $p = 0.001375$ (antagonism)	
48*	*Daphnia magna*	Carbendazim	Dissolved oxygen Acute test: 0.1–1.9 Feeding inhibition test: 2–6	48 h (in ASTM hard water + solvent < 100 μg/L) 24 h exposure (in ASTM hard water + solvent < 100 μg/L) 24 h exposure + 4 h post-exposure (in ASTM hard water + solvent < 100 μg/L)	Survival (χ^2 test) Feeding rate (χ^2 test) Feeding rate (χ^2 test)	$p = 0.0000277$ (synergism) $p = 5.59 \times 10^{-6}$ (antagonism) None	Ferreira et al., 2008
49*	*Daphnia magna*	Nickel	Temperature Acute test: 24 °C–39.5 °C Feeding inhibition test: 20 °C–24 °C	48 h (in ASTM hard water) 24 h (in ASTM hard water) 24 h exposure + 4 h post-exposure (in ASTM hard water)	Survival (χ^2 test) Feeding rate (χ^2 test) Feeding rate (χ^2 test)	$p = 0.00303$ (dose level dependency) $p = 0.000546$ (dose ratio dependency) $p = 0.000215$ (antagonism)	Ferreira et al., 2010
50*	*Daphnia magna*	Nickel	Temperature Acute test: 5 °C–20 °C Feeding inhibition test: 16 °C–20 °C	48 h (in ASTM hard water) 24 h (in ASTM hard water) 24 h exposure + 4 h post-exposure (in ASTM hard water)	Survival (χ^2 test) Feeding rate (χ^2 test) Feeding rate (χ^2 test)	$p = 2.51 \times 10^{-17}$ (dose level dependency) $p = 0.0186$ (dose ratio dependency) None	Ferreira et al., 2010
51*	*Daphnia magna*	Nickel	Dissolved oxygen Acute test: 0.1–1.9 Feeding inhibition test: 2–6	48 h (in ASTM hard water) 24 h (in ASTM hard water)	Survival (χ^2 test) Feeding rate (χ^2 test)	$p = 0.000824$ (dose level dependency) $p = 0.000589$ (antagonism)	Ferreira et al., 2010

52	*Danio rerio* embryos and larvae[c]	Diazinon	Temperature (26–33.5)	72–96 h (depending on temperature)	Multiple developmental endpoints according to the SOP for zebrafish early life stage tests (MixTox model)	None	Osterauer and Köhler, 2008
53	*Danio rerio* embryos and larvae[c]	Thiacloprid	Temperature (26–33.5)	72–96 h (depending on temperature)	Multiple developmental endpoints according to the SOP for zebrafish early life stage tests (MixTox model)	None	Osterauer and Köhler, 2008
54	*Danio rerio* embryos and larvae[c]	Imidacloprid	Temperature (26–33.5)	72–96 h (depending on temperature)	Multiple developmental endpoints according to the SOP for zebrafish early life stage tests (MixTox model)	None	Scheil and Köhler, 2009
55	*Danio rerio* embryos and larvae[c]	Chlorpyriphos	Temperature (26–33.5)	72–96 h (depending on temperature)	Multiple developmental endpoints according to the SOP for zebrafish early life stage tests (MixTox model)	None	Scheil and Köhler, 2009
56	*Danio rerio* embryos and larvae[c]	3,4-dichloraniline	Temperature (26–33.5)	72–96 h (depending on temperature)	Multiple developmental endpoints according to the SOP for zebrafish early life stage tests (MixTox model)	None	Scheil, unpublished
57	*Danio rerio* embryos and larvae[c]	Nickel	Temperature (26–33.5)	72–96 h (depending on temperature)	Multiple developmental endpoints according to the SOP for zebrafish early life stage tests (3d isoboles)	$p = 0.0025$	Scheil and Köhler, 2009
58*	*Danio rerio*	Chlorpyriphos (0–1)	Dissolved oxygen (0.81–8.63)	2 h (water)	Locomotor activity (MixTox model)	$p<0.0001$	Kienle et al., unpublished (personal comm.)
59*	*Danio rerio*	Nickel (0–15)	Dissolved oxygen (0.81–8.05)	2 h (water)	Locomotor activity (MixTox model)	$p<0.001$	Kienle et al., 2008
60	*Danio rerio*	Thiacloprid (0–10)	Dissolved oxygen (2.14–7.78)	2 h (water)	Locomotor activity (MixTox model)	$p<0.05$	Langer et al., unpublished (personal comm.)
61*	*Danio rerio*	Imidacloprid (0–50)	Dissolved oxygen (1.92–7.83)	2 h (water)	Locomotor activity (MixTox model)	$p<0.001$	Langer et al., unpublished (personal comm.)

[a] Statistical method used to analyze results: ANOVA = analysis of variance; GLM = general linear models; MixTox = model developed specifically for analysis of deviations from SA and IA models (Jonker et al., 2005).
[b] Interactions together with significance levels are reported; where tested and reported, also the type of deviation from no-interaction model is given but this should be treated with caution (see Introduction).
[c] According to the Standard Operating Procedure (SOP) for zebrafish test, 24 different endpoints are included in the test and are not reported here. For details see e.g., Scheil and Köhler (2009).
[d] Due to the nature of the study, some data are available at the time of printing as internal NoMiracle project reports only. They are available from the authors on request.

effects. For example, the survival of adult ground beetles and their larvae (Bednarska et al., 2009 and Bednarska and Laskowski, 2009, respectively) was less affected by combined effect of nickel and chlorpyriphos at lower temperatures (10 °C) than at higher (25 °C) (see Case study 2). The reproduction was most sensitive to Ni concentration at the lowest and highest temperatures (Bednarska et al., 2009), while the effect of Ni×T interaction on the proportion of emerged imagines indicated stronger toxicity of Ni at higher temperature (Bednarska and Laskowski, 2009). These results show that different endpoints may be differently sensitive to the same interaction and underline the importance of considering multiple factors in assessment of risk brought by exposure to toxic chemicals in natural conditions.

The TPM-p value evaluated across all 61 studies on interactions between chemicals and natural factors was 2.7×10^{-82}, and the more conservative calculation, with all significant p values set to 0.05 (see Methods) gave TPM-$p=3.4\times10^{-18}$. This indicates that depending on natural conditions, observed effects of toxic chemicals can indeed differ and the joint null hypothesis assuming no interactions between chemicals and natural environmental factors has to be rejected at a very high significance level. The TPM method does not allow for stating how many of the found interactions are in fact important/significant; it just informs that across all studied interactions at least one is significant (Neuhäuser, 2004). Nevertheless, given the extremely low probability of the type II error and a number of highly significant interactions in single studies, these results reinforce the conclusion outlined in the previous paragraph that natural factors commonly modify toxic effects of chemicals on organisms and, thus, must not be neglected in ecotoxicological tests, and especially in risk assessment. Because some tests resulted in very low p values, a number of interactions remain significant even after the most conservative approach — the Bonferroni correction. The straight Bonferroni correction (Holm, 1979) resulted in 19 studies (31.1%) showing significant interaction at the familywise error maintained at 5%. A slightly less conservative sequential Bonferroni (Holm, 1979) procedure gave 22 (36.1%) significant interactions between chemicals and natural factors, and the FDR method resulted in 28 significant cases (45.9%) (Table 1). Additionally, it has to be mentioned that the actual number would probably be even higher for any of the corrections applied if accurate significance levels were available for all studies considered in this meta-analysis as in few cases the significance levels were reported only as <0.05.

The interactions can take different shapes, resulting in either increased or decreased effects of chemicals at suboptimal environmental conditions. The first of the two examples (case studies) described in more detail below shows how the first-order interaction between temperature and a chemical leads to different estimated effects across the European continent. The second example concentrates on second-order interactions, indicating how complex effects of natural factors on toxicity of chemicals can be.

3.1. Case study 1: combined effect of temperature and phenanthrene on population growth rate of the potworm *E. doerjesi* (Kramarz, unpublished)

Phenanthrene (PHE) belongs to the PAHs (polycyclic aromatic hydrocarbons) family and is one of the most abundant PAHs in the environment. Contaminated forest soils may contain up to a few hundred μg PAHs per kg, in urban soils concentrations can reach a few thousand μg kg^{-1}, and phenanthrene concentrations up to 2809 μg^{-1} have been documented (Wilcke, 2000). Even higher concentrations of PAHs, up to 186 mg kg^{-1}, were reported by Krauss et al. (2000) for urban soils, and around 60 mg PHE kg^{-1}, were found by Arbabi et al. (2009) in soil contaminated with crude oil. Phenanthrene metabolism may lead to adduct formation and DNA damage (Van Brummelen et al., 1996). Acute toxicity is rarely reported in humans, fish, or wildlife, as a result of exposure to low levels of single PAH compounds. PAHs are more frequently associated with chronic risks, including cancers, which often result from exposures to complex mixtures of chronic-risk aromatics (such as PAHs, alkyl PAHs, benzenes, and alkyl benzenes), rather than exposures to low levels of a single compound (e.g., Irwin et al., 1997).

Enchytraeus doerjesi is a recently discovered species (Westheide and Graefe, 1992), easy to culture and fast growing, which makes it an ideal object for population studies and ecotoxicological tests. The study was done on laboratory cultures of *E. doerjesi* with initial density of 20 adult individuals per vial containing 30 g wet quartz sand (pH_{H_2O} 6.0, moisture 70% water holding capacity) (Kramarz et al., 2005). Each culture was exposed to one of the four phenanthrene (Sigma Aldrich, 98%) concentrations plus control (0): 100, 200, 400 or 800 mg kg^{-1}, (nominal values) at 10, 20, 25, and 30 °C. The study was done in five replicates in a full factorial design. The animals were kept in darkness and fed *ad libitum* with sterilized rolled oats replaced every second day. After 4 weeks (approximately the span of one generation) the animals were killed by pouring 3% formalin solution into the experimental vials. They were then washed out from the vials and dyed with Rose Bengal sodium salt (Sigma) to facilitate counting. Each replicate was photographed, and the potworms were counted automatically with the Cell software (Soft Imaging System GmbH).

Because we ignored the age structure of the experimental cultures, the most appropriate expression of population growth rate was the instantaneous rate of increase (r_i):

$$r_i = \frac{\ln\left(\frac{n_t}{n_0}\right)}{t}$$

where n_0 and n_t are the population sizes at the start and at the end of the experiment, respectively, and t is time in weeks. The instantaneous population growth rate was recalculated to $\lambda = e_i^r$ in order to avoid negative numbers to allow for logarithmic transformations.

The significance of the factors and the interaction between PHE and temperature (T) for the population growth rate λ was tested by fitting the second-order equation containing terms representing main effects (β_1, β_2), second-order interaction (β_{12}), and squared effects (β_{11}, β_{22}) (Statgraphics Centurion package, Manugistics Inc.). Logarithm of PHE + 100 was used rather than the raw data in order to meet the assumption of normal distribution:

$$\lambda = \beta_0 + \beta_1 \times \log_{10}(PHE + 100) + \beta_2 \times T + \beta_{12} \times \log_{10}(PHE + 100) \times T + \beta_{11} \times (\log_{10}(PHE + 100))^2 + \beta_{22} \times T^2$$

When estimating the model, only factors significant at $p \leq 0.05$ were retained in the final equation.

The estimated response surface was used to produce risk scenarios for the European continent, presented as maps showing population growth rate (λ) predicted at area-specific average monthly maximum temperatures for April at two different pollution levels: 200 and 800 mg PHE kg^{-1} soil. Although the concentrations used in this procedure exceed those found for PHE in soils, they are in the range of total PAHs concentrations registered in polluted areas (Wilcke, 2000). The maps are thus meant to represent potential effects of chronic exposure to total PAHs in soil. The calculated population risks were divided into four clear categories to make the maps easier to read: areas where populations cannot persist ($\lambda = 0$) are painted red, populations going towards extinction ($0 < \lambda < 1$) are shown in orange, those at the critical population growth rate ($\lambda = 1.0$) in green, and areas where populations are able to maintain positive population growth rate ($\lambda > 1$) are marked blue. For a comparison, maps showing purely temperature effect, that is with no PHE pollution, are also shown. The climatologic data were obtained from WORLDCLIM

Version 1.4 (http://www.worldclim.org). The maps were generated using Raster Calculator tool implemented in ESRI ArcGIS 9.3 software.

3.1.1. Case study 1 — results

Both temperature and phenanthrene affected population dynamics (λ) of *E. doerjesi* significantly. The estimated response surface was described by the following equation:

$$\lambda = 3.91 + 0.364 \times T - 3.69 \times \log_{10}(PHE + 100) + 0.937 \times (\log_{10}(PHE + 100))^2 - 0.129 \times T \times \log_{10}(PHE + 100).$$

The growth rate increased with temperature ($p<0.0001$), and the animals practically did not reproduce at 10 °C. Phenanthrene did not increase mortality at this temperature. As a result, at this temperature population numbers remained approximately stable throughout the experiment irrespectively of the phenanthrene treatment. However, at higher temperatures, at which the animals were able to reproduce successfully, phenanthrene caused a significant decrease in population growth rate and the overall effect of phenanthrene was highly significant at $p<0.0001$ (Fig. 1). The fact that phenanthrene effects were different at different temperatures was reflected in the significant interaction between the two factors for the population growth rate ($p<0.0001$; Fig. 1). Using the relationship between population growth rate and temperature at each PHE concentration we calculated critical temperatures below which the potworms were not able to maintain positive population growth rate at specific PHE treatments. This temperature was ca. 6.8 °C in control cultures, and 10.7 °C at 200 mg kg^{-1}. However, at 800 mg kg^{-1}, the potworms maintained $\lambda>1$ below ca. 11.3 °C but not above this temperature. Thus, the relationship between population growth rate and combined effect of PHE and temperature was highly non-linear and impossible to predict without experimental data.

Maps of the predicted risk scenarios (Fig. 2) indicate how population growth rate changes across Europe with temperature increasing southward, both with and without assumed phenanthrene pollution. It has to be stressed that the maps have been generated for illustrative purposes only and they are not meant to depict any actual risk. Nevertheless, they allow to show that the predicted ecotoxicological risk for an organism not only changes due to the simple relationship between temperature and physiological performance of ectotherms but the interaction between PHE and temperature can even reverse the relationship at certain combinations of the two factors. This can be seen when comparing scenarios for 200 and 800 mg kg^{-1}, on Fig. 2. The population growth rate at 200 mg PHE kg^{-1} shows a relatively simple relationship with temperature: on top to the overall decrease in λ with decreasing temperature, there is a clear decrease in population growth rate due to pollution with PHE — the trend closely resembling the one for pollution-free environment. Despite the pollution, populations of the potworms can still maintain positive growth rate across large areas of central and southern Europe. However, at 800 mg kg^{-1}, the trend reverses: population growth rate decreases with increasing temperature resulting in negative population growth rate over most of the southern Europe and in large areas of its central part (Fig. 2). We do not know the actual mechanism behind this interaction between PHE and temperature and can only speculate that high temperatures may increase PHE transfer to animals and its biochemical activity, while low temperatures may act protective through very low activity of the animals (and, hence, probably also low exposure) and thanks to decreasing biochemical activity of PHE itself. This result certainly deserves more detailed biochemical studies as well as experiments on more species.

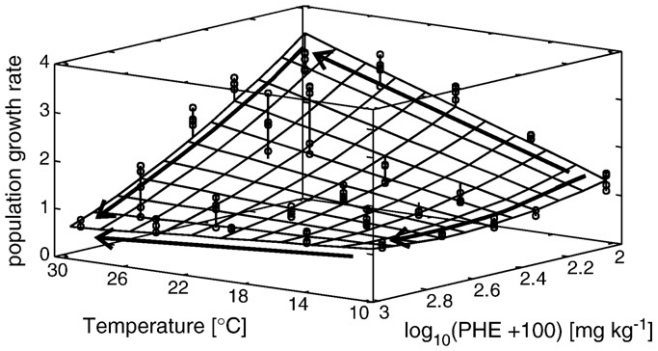

Fig. 1. The three-dimensional plot showing how the population growth rate (λ per week) of potworms (*Enchytraeus doerjesi*) depends on phenanthrene and temperature. Arrows indicate the evolutions of the response with increasing temperature and PHE concentration. Note that the relationship with phenanthrene concentration gets steeper with increasing temperature and that at the highest phenanthrene concentration the temperature effect reverses ($R^2 = 79\%$, $p<0.0001$).

3.2. Case study 2: second-order interactions between nickel, chlorpyriphos and temperature in the ground beetle Pterostichus oblongpunctatus (after Bednarska et al., 2009 and Bednarska and Laskowski, 2009)

The second-order interactions of three or more factors have rarely been studied (Chen et al., 2004; Heugens et al., 2006), mostly due to elaborate experimental design and complex interpretation of higher-order interactions. In the NoMiracle project interactions between nickel, chlorpyriphos (CPF) and temperature were studied in the ground beetle *P. oblongpunctatus* (Coleoptera: Carabidae). For the purpose of this article the experimental designs and results of both experiments are summarized briefly below.

Separate experiments were run on adult beetles and on larvae. Adult beetles were randomly allocated to three experimental groups fed food contaminated with 5000 or 10,000 mg Ni kg^{-1}, or uncontaminated. After 4 weeks, the beetles were dosed topically with 40 or 80 ng active ingredient (a.i.) of CPF dosed in 1 μl of acetone or with 1 μl of pure acetone (solvent control). The beetles were then transferred to one of three temperatures: 10, 20 or 25 °C. After 48 h those beetles which survived CPF treatment were coupled according to the treatment, and each pair of beetles was kept in a separate box, four replicate pairs per treatment. The recorded endpoints were the lifetime (followed for 134 days since the pesticide application) and the reproduction rate expressed as the number of eggs produced per female. For more details see Bednarska et al. (2009).

The newly hatched larvae were transferred individually to 30 ml plastic vials with moistened peat (80% WHC) contaminated with 0, 0.5, 1 or 2 mg a.i. CPF kg^{-1}, dry weight. Then, larvae were randomly assigned to one of three artificial foods spiked with 0, 600 or 1200 mg Ni kg^{-1}, dwt and were cultured at three different temperatures, 10, 20 or 25 °C, in darkness at 75% RH. Each individual larva was treated as a replicate, with 10 to 18 replicates per treatment. Altogether, 492 larvae were used in the experiment. The experiment was ended after 125 days, when all larvae had either pupated or died. The recorded endpoints were the lifetime followed for 125 days and the proportion of imagines emerged in each treatment (Bednarska and Laskowski, 2009). The possible interactions between chemicals and temperature were studied in a full factorial test design and analysed with a general linear model (GLM).

3.2.1. Case study 2 — results

The results revealed significant second-order interactions in survival of both larvae ($p=0.01$) and adult beetles ($p=0.006$). In contrast, no second-order interactions were found in the fecundity (number of eggs per female) or in the development success rate (proportion of imagines emerged from larvae). In the latter two cases only the first-order interactions were identified, namely Ni × *T*

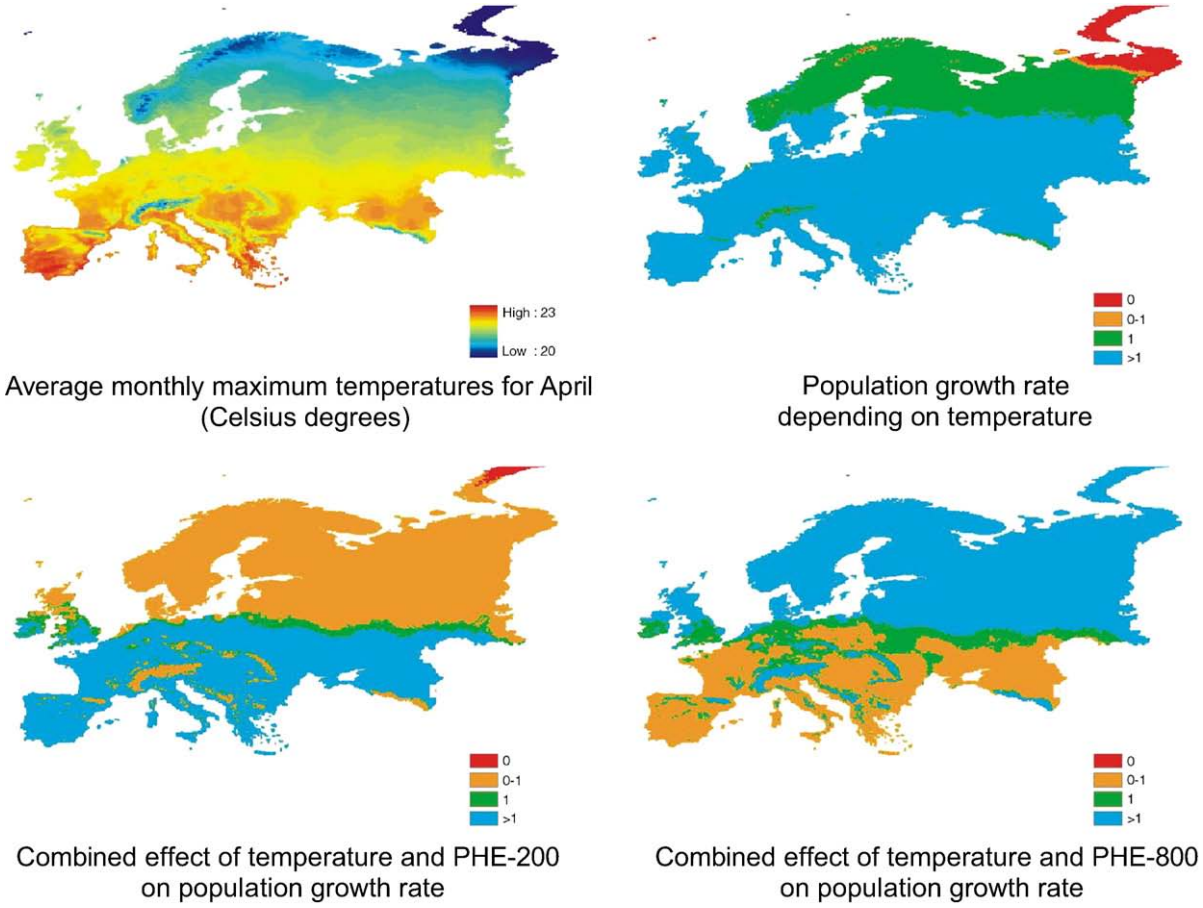

Fig. 2. Maps showing average maximum April temperatures and predicted effects of temperature alone and combined with phenanthrene at two assumed soil pollution levels, 200 and 800 mg kg^{-1} (PHE-200 and PHE-800), on population growth rate (λ) of the potworm *Enchytraeus doerjesi* across European continent. Effects on λ are shown in four colours, depicting extinct populations (λ = 0; red), shrinking populations (0<λ<1; orange), populations at critical population growth rate (λ = 1; green), and growing populations (λ>1; blue). For details on how the maps were produced see Case study 1.

($p = 0.03$) for number of eggs per female, and Ni × T ($p = 0.03$) and CPF × T ($p < 0.0001$) for development success rate.

The second-order interaction between Ni, CPF and T in adults shows how temperature may affect the interaction between the two chemicals (Fig. 3). Thus, in unpolluted environment (Ni-0, CPF-0), temperature did not affect the survival rate and animals at all temperatures were able to survive until the end of the experiment: the model-estimated survival time is 138 days (actual length of the experiment was 134 days). In the beetles not treated with CPF, the exposure to Ni at 10,000 mg kg^{-1}, decreased the estimated survival time to 67 days at 10 °C but even more, to 32 days, at 25 °C (Fig. 3).

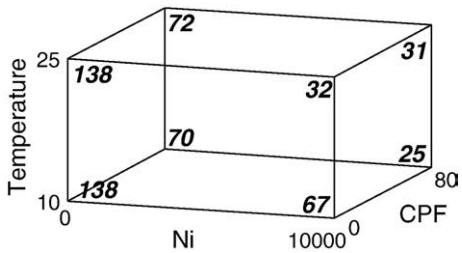

Fig. 3. The cube plot showing estimated effects of nickel (Ni), chlorpyriphos (CPF) and temperature on life time of the adult ground beetles *Pterostichus oblongopunctatus*. Numbers on axes outside the cube show the minimum and maximum values of the factors used in the experiment (Ni — mg kg^{-1}; CPF — ng a.i. per beetle); the numbers typed in boldface italics in corners inside the cube show the estimated life time of the beetles (days) at specific combinations of the three factors (plot based on data from Bednarska et al., 2009).

When the beetles were treated with CPF but not with Ni, the estimated survival time decreased to the approximately same value, 70–72 days, at both the lowest and the highest temperature. There was thus no temperature effect in beetles not exposed to any toxicant or exposed solely to CPF, but those exposed only to Ni survived substantially better at low than at high temperature. What is even more interesting, when the beetles were exposed simultaneously to the highest Ni concentration in food (which alone resulted in substantially higher effect at 25 °C then at 10 °C) and CPF, the temperature effect disappeared or was even reversed: at the highest Ni and CPF treatments the estimated life time was 25 days at 10 °C and 31 days at 25 °C. With this relatively small difference in survival time we cannot exclude however that this reversal was actually an artifact resulting from fitting the complex surface to a limited data set.

Because it can be argued that such high concentrations of Ni in food as used in this experiment could exceed environmentally realistic scenarios, it is worth mentioning that in the vicinity of smelters, Ni concentrations in soils may exceed 6500 mg kg^{-1}, (Stefanowicz et al., 2008), and concentration as high as 22,000 mg Ni kg^{-1}, was found in smelter-contaminated soil by Everhart et al. (2006).

4. Conclusions

The review of 61 studies on interactive effects of toxic chemicals and natural environmental factors showed that in approximately every second case natural conditions significantly modified effects of toxicants on tested organisms. The significant interactions included also second-

order interactions, indicating that not only toxicity of single chemicals but also interactions among toxicants can be affected by natural conditions. The results emphasize the necessity of incorporating natural environmental conditions, characteristic for different geographic regions, in ecological risk assessment. We advocate designing such tests for the second-tier ecological risk assessment procedures.

The two case studies show that interactions between toxic chemicals and temperature are complex and difficult to predict. In the first case (potworms), the combined effect of temperature and PHE changed in a non-linear fashion with temperature, hence the predicted effects of pollution with this chemical differed vastly across Europe, depending on the area-specific combinations of phenanthrene concentration and temperature. The second case study revealed that also higher-order interactions between chemicals and temperature are possible and can be significant — the rather poorly known phenomenon, certainly deserving further studies.

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