Ecotoxicology and Environmental Safety 134 (2016) 196–203

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Modification of nickel accumulation in the tissues of the bank vole *Myodes glareolus* by chemical and environmental factors

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ARTICLE INFO

Article history:
Received 5 April 2016
Received in revised form
18 August 2016
Accepted 22 August 2016

Keywords:
Bank vole
Nickel accumulation
Chlorpyrifos
Temperature
Interactions
Body and tissue masses

ABSTRACT

In a full factorial laboratory experiment, the effects of temperature and two chemical stressors (nickel and chlorpyrifos) on the accumulation of nickel in the liver and kidney of bank voles were studied. The nine-week experiment consisted of three periods: acclimatisation (3 days), intoxication (6 weeks) and elimination (3 weeks). During the main intoxication phase the animals were orally exposed for 42 days to different doses of nickel (Ni) (0, 300 and 800 mg/kg food) or chlorpyrifos (CPF) (0, 50 and 350 mg/kg food) or a mixture of both chemicals. Additionally, animals from each chemical treatment were divided into subgroups assigned to three temperatures: 10, 20 or 30°C. The highest concentrations of nickel were found in the testis, but there were no statistical effects of studied factors on this tissue. The nickel concentrations were higher in the kidney than in the liver of the bank voles. Nickel levels in the livers were influenced by Ni concentration in the food during intoxication time and additionally by interactions between Ni, temperature and day of exposure during elimination. The kidney concentrations of nickel depended on the level of nickel exposure but also on the interactions of the nickel with other factors: temperature, chlorpyrifos, day of exposure. This influence was observed only during the intoxication phase. The body mass and liver and kidney masses of the animals were affected both by the nickel concentration in the food and by the temperature.

The capsule abstract: Ni in the tissues depended on the interactions between the factors: Ni, temperature and other. The body, liver and kidney masses were affected by both Ni in the food and by the temperature.

1. Introduction

The problems addressed in the paper i.e. the complex effects of chemical and non-chemical environmental factors on human and animal populations has been shown to be important, especially for relevant environmental risk assessment. The toxicological tests recommended by the Organisation for Economic Co-operation and Development (OECD) and US EPA (United States Environmental Protection Agency) provide information only on the effects of a single toxicant on various lifespan endpoints, which is not realistic from an environmental point of view because organisms are exposed in nature to various mixtures of chemicals, which can act in various ways vis a vis producing additive, non-additive or synergistic effects (Elumalai et al., 2002; Cooper et al., 2009; Baylay et al., 2012; Pérez et al., 2013). What is more, the characteristics and toxicity of chemicals may be modified by physical environmental factors, such as temperature or humidity, which can additionally influence the organism response to the toxicant's intoxication (Schiedek et al., 2007 review; Świergosz-Kowalewska et al., 2014; Ferreira et al., 2008). This was proven for some organic and nonorganic pollutants, and mostly for invertebrate species (Bednarska and Laskowski, 2009; Holmstrup et al., 2010; Osterauer and Kohler, 2008). Some factors e.g. temperature can modify both the chemical toxicity and animal response, some e.g. humidity may influence only the chemical toxicity (Gordon, 2005; Talent, 2005 review). Holmstrup et al. (2008) showed that exposure to chemical stress, in this case Hg, increased the sensitivity of *Folsomia candida* to cold shock and vice versa cold shock increased the susceptibility to Hg, even at temperatures that did not have lethal effects under the control conditions (without metal exposure).

Additionally, climate change, rising global temperature and frequency of extreme temperatures observed over the last few decades has strengthened interest in the interactions between temperature and contaminants in the contexts of organismal response (Schiedek et al., 2007). As many authors point out, studies

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http://dx.doi.org/10.1016/j.ecoenv.2016.08.015
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without such a complex approach do not give results, which can be extrapolated to environmental conditions.

The literature search confirmed that during the last few years not only more researchers have perceived the need for complex investigation of various stressors (review by Holmstrup et al., 2010) but also more data are available on this topic (Broerse and van Gestel, 2010b; Boyd, 2010; Dondero et al., 2010; Owojori and Reinecke, 2010; Lister et al., 2011). However, in most experiments the researchers study only the effects of chemical mixtures without physical stress (Broerse and van Gestel, 2010a; Green and Walmsley, 2013). Furthermore, most of the investigations are performed on invertebrate species, due to easy maintenance of large number of individuals (Kim et al., 2010), and there is a considerable lack of data for vertebrate species. Of course, many aspects of chemical toxicity may be tested with invertebrate species, because their response would be similar to vertebrate ones, but for the effects of physical factors, like temperature, this response might be different and not so obvious due to energy allocation into thermoregulations (Gordon, 2005; Begon et al., 2008).

Also, the large NoMiracle project, oriented on the effects of both chemical and non–chemical factors on various organismal end points, was conducted mainly on invertebrate species (Kienle et al., 2009; Løkke 2010; Løkke et al., 2013; Laskowski et al., 2010). As a part of this project, we decided to study the same group of factors: temperature (T), nickel (Ni) and chlorpyrifos (CPF) in full factorial experiment, but using the bank vole as the representative of vertebrates. We chose the bank vole, *Myodes (=Clethrionomys) glareolus* because it is very common in various natural environments and may serve as a model species for rodents. Our main experimental question concerned the presence or not of the interactive effects of the studied factors on nickel accumulation in different tissues of this species.

The main chemical chosen for our study was nickel, which is a not such a common environmental pollutant as cadmium or lead; however, its elevated abundance in some mining and industrial areas is noticeable and causes toxicological problems (Denkhaus and Salnikow, 2002; Everhart et al., 2006; Henderson, 2012). For example, Martiniaková et al. (2012) reported high concentration of this metal (up to 14 mg/kg dry weight tissue) in the femur of small rodents (*A. flavicollis*, *A. sylvaticus*, *M. glareolus* and *M. arvalis*) collected at contaminated sites in Slovakia. Even higher Ni concentrations were detected by Tovar-Sánchez et al. (2012) in the bone tissues of *Baiomys musculus* and *Peromyscus melanophrys* – up to 55 mg/kg dry weight. What is more, nickel presence in human common surroundings (together with dust particles) and its toxic effects are also recognised. This is due to using many products containing some amounts of this element, for example steel cooking pots or jewellery (Cempel and Nikel, 2006) but also some food products and cosmetics (Akinyele and Shokunbi, 2015; Bocca et al., 2014). The oral and inhalation exposures to Ni within EU countries were estimated in detail by De Brouwere et al. (2012) and Buekers et al. (2015), respectively. An extensive review concerning acute oral toxicity of different nickel compounds was published by Henderson et al. (2012). Whereas Harasim and Filipek (2015) provided information on nickel presence in the environment as well as its toxicity.

The second chemical chosen for our experiment was chlorpyrifos, which is a representative of the large group of phosphoorganic pesticides use for pest protection. Environmental contamination by this compound and its toxic effects is well known and does not require special supporting information (Barron and Woodburn, 1995; Mileson et al., 1998; Cleveland et al., 2001; Cochran. 2002; USEPA, 2002; Al-Badrany and Mohammad, 2007; Agrawal and Sharma, 2010; Krieger, 2010).

Bearing in mind that the estimation of uptake and accumulation of various pollutants in a context of acting physical factors is crucial, we chose temperature as a third stressor: values within (20°C), below (10°C) and above (30°C) a rodent neutral temperature zone. Such ambient temperatures for endothermic species may have an impact on the metabolism rate and detoxification processes, and can modify the overall toxicity of acting chemicals. A good example concerning the negative effect of thermal stress on detoxification processes connected with Ni exposure in *Mytilus galloprovincialis* was provided by Attig et al. (2014). Both catalase (CAT) activity and glutathione levels were decreased at the highest temperature of 26°C. Similar results were obtained for the same species by Banni et al. (2014), where catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST) were significantly decreased as a result of exposure to Ni and a higher temperature (26°C) for 72 h.

Our hypothesis was that the two additional to nickel stressors - chlorpyrifos and temperature – would modify the accumulation of nickel in the tissues of bank voles, and that temperature would be an important factor influencing metal toxicokinetics and the overall effects of chemicals on the toxified organism. In the paper three endpoints – the level of nickel in the tissues, body mass and organs masses – are reported, providing the possibility to compare the response of different tissues and the sensitivity of endpoints.

2. Material and methods

2.1. Object of the study

The I Local Ethics Committee for Research on Animals at Jagiellonian University approved all the procedures used in this experiment.

The experiment was performed on 495 bank vole males, four- to six-months-old, bred for 5 generations in a breeding laboratory after collecting from a wild field population (the details of the breeding regime are in the paper by Sadowska et al. (2009)). The laboratory population remained non-inbred (the inbreeding coefficient was 0.007). The variance in age was the same in all treatments. The body mass of the animals ranged from 16.5 to 36.8 g at the beginning of the experiment.

2.2. Experiment design

The experiment consisted of three periods: acclimatisation (3 days), intoxication (42 days) and elimination (21 days), giving nine-week exposure. The voles were assigned to 27 treatments with various combinations of studied factors: temperature (10, 20 or 30°C), nickel concentration (0, 300 and 800 mg/kg dry weight) and chlorpyrifos (0, 50 and 350 mg/kg dry weight) in the food. Only during the intoxication phase, the animals were orally exposed to different concentrations of nickel or chlorpyrifos, or a mixture of both chemicals, and additionally to different temperatures. During the acclimatisation and elimination phases, the animals were given clean food and were exposed only to different temperatures according to earlier assignments. The voles were kept individually in standard animal cages in an animal room under a 12-h light/dark regime and were given standard food pellets (Animal Food Factory A. Morawski, Poland) and water *ad libitum*.

The voles were randomly assigned to 9 Ni or/and CPF treatments (all concentrations reported for dry weight of food, N=number of individuals per treatment):

Control – 0 mg Ni and 0 mg CPF/kg (N=63).
Ni0/CPF50 – 0 mg Ni and 50 mg CPF/kg (N=54).
Ni0/CPF350 – 0 mg Ni and 350 mg CPF/kg (N=54).
Ni300/CPF0 – 300 mg Ni and 0 mg CPF/kg (N=54).
Ni800/CPF0 – 800 mg Ni and 0 mg CPF/kg (N=54).

Ni300/CPF50 – 50 mg CPF and 300 mg Ni/kg (N=54).
Ni800/CPF50 – 50 mg CPF and 800 mg Ni/kg (N=54).
Ni300/CPF350 – 350 mg CPF and 300 mg Ni/kg (N=54).
Ni800/CPF350 – 350 mg CPF and 800 mg Ni/kg (N=54).

Additionally, the animals from each treatment listed above were assigned to 3 subgroups, which were kept in rooms at three different but constant temperatures: 10, 20 or 30°C.

2.3. Measurements during the experiment

The body mass of each vole was recorded at the 0 and 42 day of the experiment. The animals (3 per experimental day) were sacrificed at 0, 5, 10, 20 and 42 day of the intoxication phase and at the 49 and 63 day of the elimination phase. The livers, kidneys and testes were obtained and the livers and kidneys weighed. All the collected tissue samples were preserved at −70°C for chemical analysis.

2.4. Food preparation

Standard food pellets (Animal Food Factory A. Morawski, Poland) contained 21.5% protein, 4.0% lipids and 3.7% fiber in dry mass. Contaminated food was produced by mixing 1 kg of standard food powder with 0.5 L of water and 0.5 L of rape seed oil. Nickel chloride (Sigma Aldrich) and chlorpyrifos (O,O-Diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) (Cheminova A/S, Denmark) were dissolved in the water or oil, respectively, to give the desired experimental concentrations. Fresh food was prepared every 2 weeks from the beginning to end of the intoxication period and kept in closed plastic containers in a dark room at −4°C to protect the chlorpyrifos from degradation. Degradation half-life of chlorpyrifos is usually around 4–10 weeks in soil and water, so the applied storage conditions protected it from degradation.

2.5. Chemical analyses

For Ni analyses, the tissues samples were dried at 60°C and then wet-digested in concentrated nitric acid (Suprapur grade, Merck, Germany) according to standard mineralisation procedure. Concentrations of nickel were determined by atomic absorption spectrometry (Analyst 800, Perkin-Elmer). Certified reference material (DORM-2; Dogfish Muscle Certified Reference Material for Trace Metals; NRC, Ottawa, Canada) was used to check the analytical precision. The obtained mean value was 21.77 mg/kg ± 3.07 SD, while the certified value was 19.4 ± 3.1 μg Ni/kg. Nickel concentration was expressed in mg/kg dry weight.

2.6. Statistical analysis

Arithmetic mean and standard errors (± SE) for each data set concerning Ni concentrations in the tissues were calculated. The data of Ni levels were not distributed normally, so logarithmically transformed data were used (Zar, 1999) for further analyses. Factorial ANOVA and the *a posteriori* Tukey honest significant difference test for unequal numbers of replicates ($p < 0.05$) were used to test, separately for the intoxication and elimination phase, the effects of nickel, chlorpyrifos, temperature and exposure time on nickel accumulation in the tissues (Statistica 9.0). The effects of the studied factors on body mass changes between the 1st day and 42nd day of intoxication were calculated with ANCOVA, where the body masses of animals at the 1st day were taken as a cofactor.

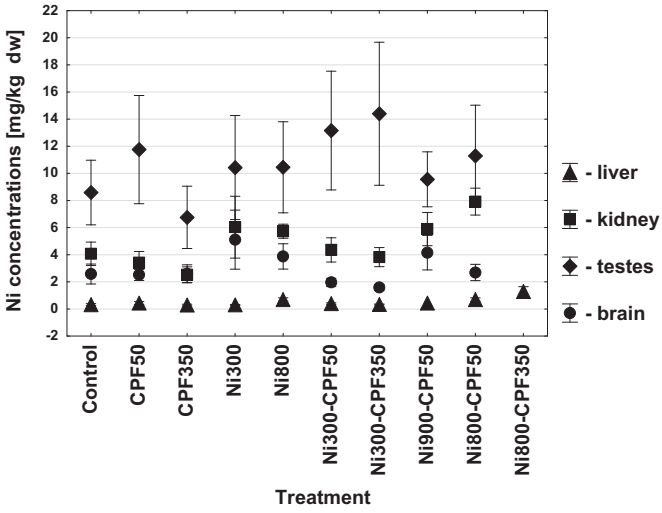

Fig. 1. Nickel concentrations [mg/kg dry weight] in the tissues of bank voles exposed to different concentrations of Ni, CPF and a mixture of both chemicals. Arithmetic means ± standard error. The data for brain tissue after Świergosz-Kowalewska et al. (2014).

3. Results

3.1. Nickel accumulation in the tissues

The highest concentrations were found in the testes and the lowest in the livers of the bank voles (Fig. 1). The brain nickel concentrations (after Świergosz-Kowalewska et al., 2014) were higher than in the liver. Generally, the levels of nickel in the tissues were not very high; the highest average value was about 14 mg/kg dw in the testes. Nickel accumulation in the livers of the bank voles was significantly nickel exposure-dependent during the intoxication phase ($p < 0.0001$) (Fig. 2A). Temperature in interaction with Ni concentration in the food and the day of exposure significantly influenced nickel levels in the livers during the elimination phase of experiment ($p=0.042$).

Ni concentrations in the kidneys during the intoxication phase depended on Ni exposure and nickel in interaction with temperature, CPF and day of exposure (Table 1, Fig. 2B). The effect of interaction of Ni in the food and temperature was significant at $p=0.022$ (Fig. S1). There were no noted effects of the studied factors on Ni accumulation in the testes.

3.2. Body mass changes

The body masses of the animals at day 0 of the intoxication phase did not differ ($p > 0.05$) between the 27 treatments. The body masses of the animals during the intoxication phase were influenced by Ni concentrations in the food ($p=0.023$) (Fig. 3A) and temperature ($p < 0.001$) (two degree level of interaction) (Fig. 3B). There were no effects of interactions between the studied factors on the body mass of the animals. Generally, at the end of the intoxication phase (day 42), the animals from the group exposed to highest Ni concentrations in the food had the highest decrease of body mass in comparison to animals exposed to 0 and 300 mg Ni/kg food. The temperature of 30°C had the highest effect on body mass changes; the animals exposed to this temperature significantly increased body mass during the intoxication phase (Fig. 3B, S2). The animals exposed to 10°C had a higher decrease of body mass than the animals exposed to 20°C but the difference was not statistically significant. The combined effect of chemical exposure at the various temperatures is presented in Figs. 4 and S2.

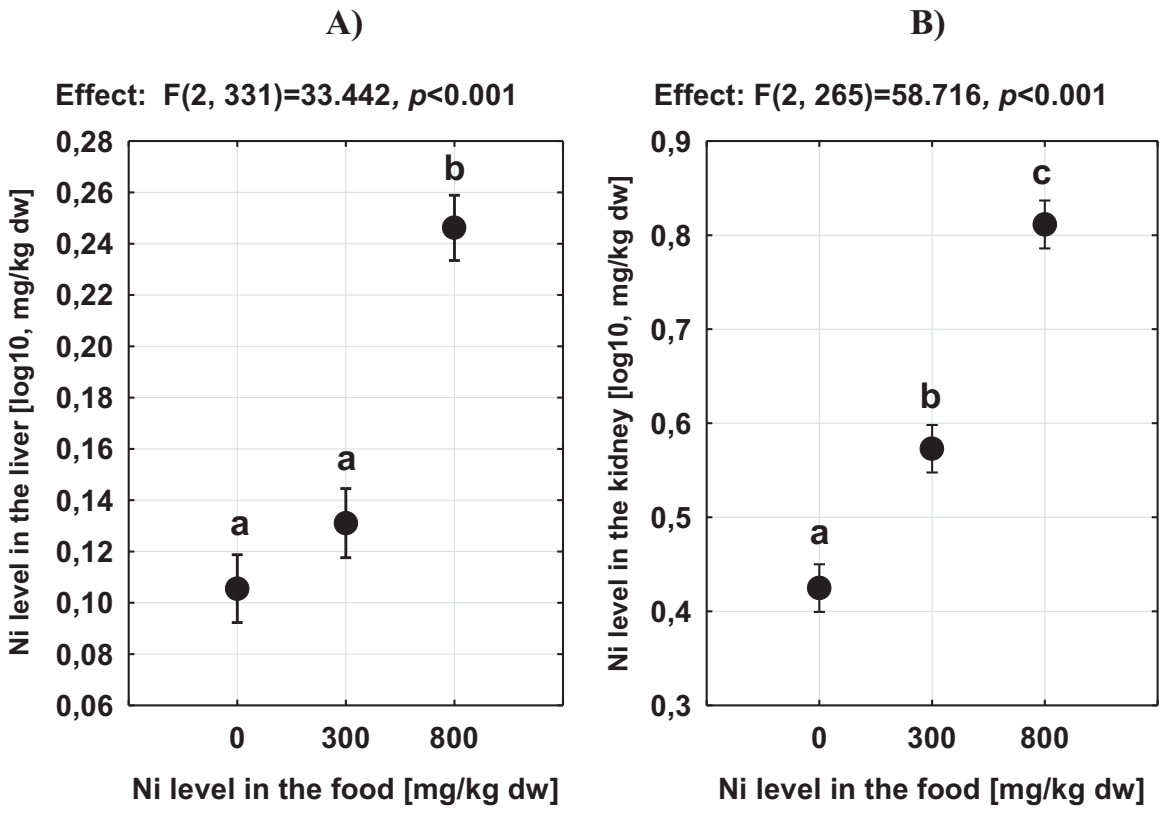

Fig. 2. Effects of nickel in the food on Ni concentrations in the livers (A) and kidneys (B) of bank voles during the intoxication phase. Arithmetic means ± standard error. The overall effect significant at $p < 0.001$ (Tukey test). The same lowercase letters means no significant differences between Ni concentrations in the food.

Table 1
Summary of multifactor analysis of variance for effects of studied factors on nickel accumulation in the kidney of bank voles. Only factors significant at $p \leq 0.05$ are reported.

Factor	Intoxication phase		Elimination phase	
	F-ratio	p-value	F-ratio	p-value
Ni	58,716	< 0,001	5,952	0,003
CPF			3,527	0,031
Ni*T	2,917	0,022		
Ni*T*D	2,345	0,007		
Ni*CPF*D	2,488	0,004		
Ni*CPF*T*D	2,889	< 0,001		

Ni – nickel; CPF – chlorpyrifos; T – temperature; D – day of exposure.

3.3. Organ masses

Temperature was a factor that affected the liver and kidney masses of animals (standardised values). After the intoxication phase, the lowest liver and kidney masses were noticed in animals exposed to the highest temperature 30°C (Fig. 5). The same pattern was observed for the liver and kidney when the effect of two factors, Ni in the food and temperature, was taken into account.

4. Discussion

The number of chemicals produced and released into the environment is continually increasing because the older ones, which are withdraw from circulation, are quickly replaced by new ones. Their lifetime and toxicity is tested before use, but mostly with usage of a limited number of laboratory animals, many often from selected species lines with low genetic variation. Moreover, the OECD tests recommended for such purposes are performed with a single substance, when the thermal condition of the experiment is stated precisely within a species neutral zone (cf. recommendations for OECD Guidelines for the Testing of Chemicals, http://www.oecd.org/chemicalsafety/testing/). In reality, these testing conditions are far removed from those observed in the natural environment, where organisms are exposed to a mixture of toxic substances differing in properties and mode of actions, that additionally may be modified by physical environmental factors e.g. temperature, humidity, salinity and others. For sure, data obtained from OECD tests are useful for a first estimation of substance toxicity, but only very roughly reflect real environmental conditions. Our investigation was designed with the intention of being more realistic than current toxicological tests by merging chemical factors with different modes of action and physical factors into one experiment.

It is well known that when an organism is exposed to one stressor, the whole accessible energy is allocated into the activated, specific for the pollutant, detoxification processes. Exposure to additional stressors such as chlorpyrifos, which is metabolised in the organism, cause a necessity to divide the energy budget across several detoxification processes, one at the cost of the other. Therefore, the exposure to additional chemical stress can result in an elevated accumulation of one of the chemicals as a result of depression of the processes connected with its detoxification. As we know, temperature – a physical stressor – can modify not only the chemical properties of chemicals in the environment through degradation or mobilisation, but it is also a strong factor influencing the physiological processes in an organism. Based on this assumption, we expected that exposure to different levels of used factors and exposure to their combinations would modify the uptake, elimination and, in consequence, the tissue distribution of nickel.

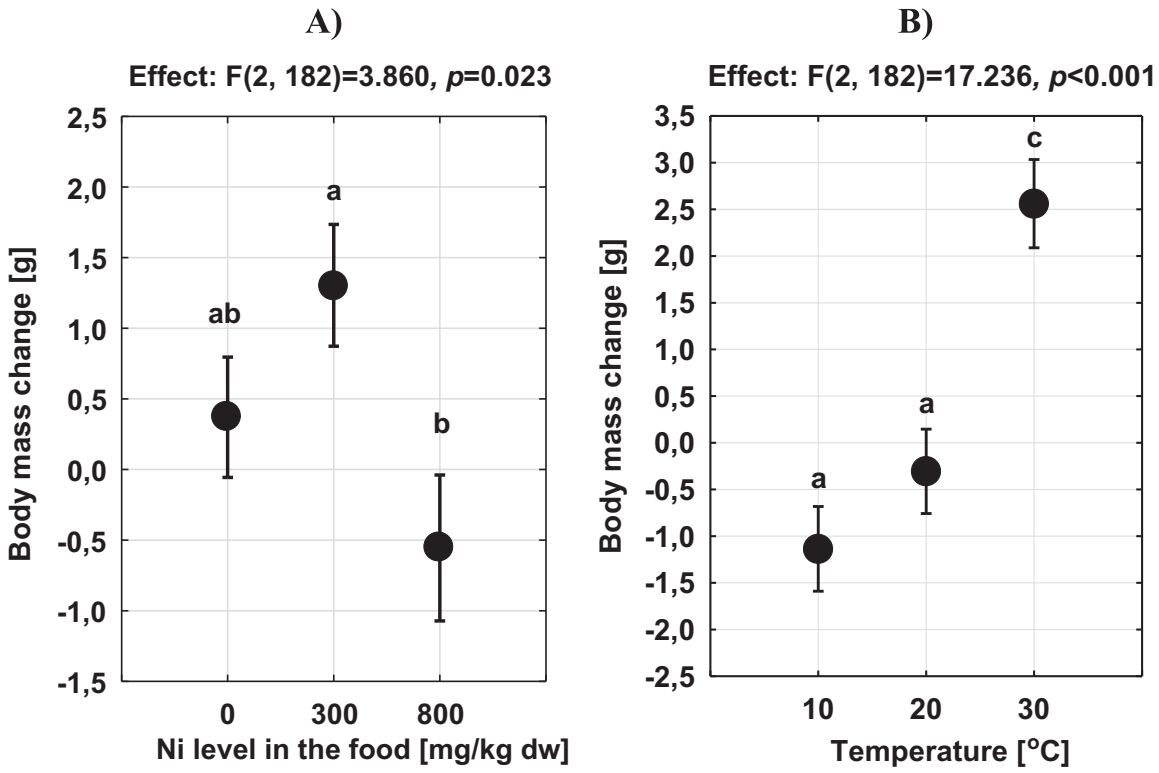

Fig. 3. The effects of Ni in the food (A) and ambient temperature (B) on body mass change measured at 42nd day of the intoxication phase (results of ANCOVA, body mass at day 0 as covariate). Arithmetic means ± standard error. The same lowercase letters means no significant differences between treatments (results of the Tukey test).

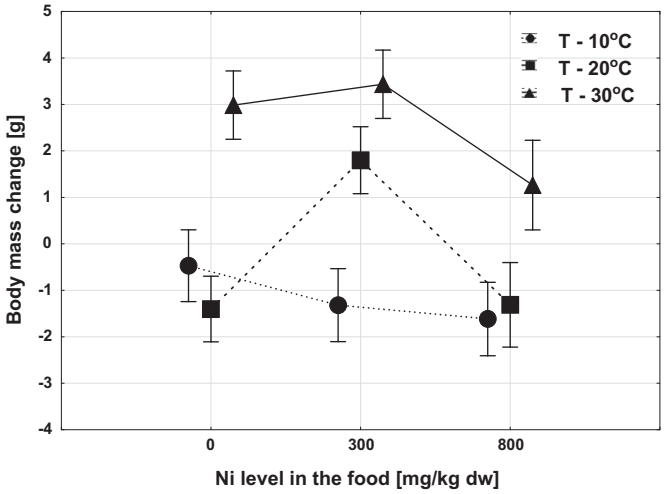

Fig. 4. Combined effects of Ni in the food (A) and ambient temperature (B) on body mass change measured at 42nd day of the intoxication phase (results of ANCOVA, body mass at day 0 as covariate). Arithmetic means ± standard error.

Our hypothesis was partially confirmed. Even when the bank voles were exposed to different concentrations of nickel in their food, up to 800 mg Ni/kg food, the tissue concentrations were not very high, reaching a maximum of 14 mg/kg dry weight tissue, and the highest concentration were found in the testes of the studied animals during the intoxication phase (Fig. 1). Lower than that Ni mean concentrations were found in succession in the kidney, brain and liver. Nickel, as metal, it is not metabolised in the organism and its uptake and tissue accumulation depends on several external and internal factors: nickel exposure concentration, chemical form and its solubility, rate of tissue absorption and physiological condition of the organism. These generally low concentrations of nickel in the tissues of the bank voles meant that the organism activated defensive processes connected with lowering of metal absorption in the intestine, which protected the animals from high metal accumulation. Such low levels of Ni in the tissues of the studied animals speaks volumes, not only in terms of the low uptake but also the quick elimination of this metal from the organism. The higher level of nickel in the kidney than in the liver proves the hypothesis about intensive elimination of this metal from the body. As it is known, the main route of nickel excretion is via the urine (Goyer, 1996), thus it is obvious that during exposure the elevated concentrations were observed in the kidneys of the bank voles (Fig. 1, data concerning brain Ni concentrations after Świergosz-Kowalewska et al. (2014)).

Similar data, the highest Ni levels being in the rat kidney tissues (10 times higher than in the liver after exposure to 1200 ppm of nickel), were obtained in the experiment by Cempel and Janicka (2002). The Ni concentrations reached 8 mg/kg wet weight of the kidney. Similar findings, the highest concentrations of Ni being in the rat kidney, were also noted by Hou et al. (2011) after intraperitoneally injection with 640 kBq/kg of ^{63}Ni. This nickel hard absorption from the intestine and intensive excretion into the urine (Goyer, 1996) observed for the studied bank voles were also found in other experiments. Very low Ni levels – almost close to the detection limit and similar in all the studied tissues – were noted by Bocio et al. (2005) in their study on the human tissues of a population living in the vicinity of a new hazardous waste incinerator (HWI) in Constantí (Tarragona County, Spain) over a period of 10 years or more.

Comparing our results to those obtained by other authors (Outridge and Scheuhammer, 1993; Martiniaková et al., 2012; Tovar-Sánchez et al., 2012; Gathwan et al., 2013) for rodent species, we could expect that the obtained tissue concentrations

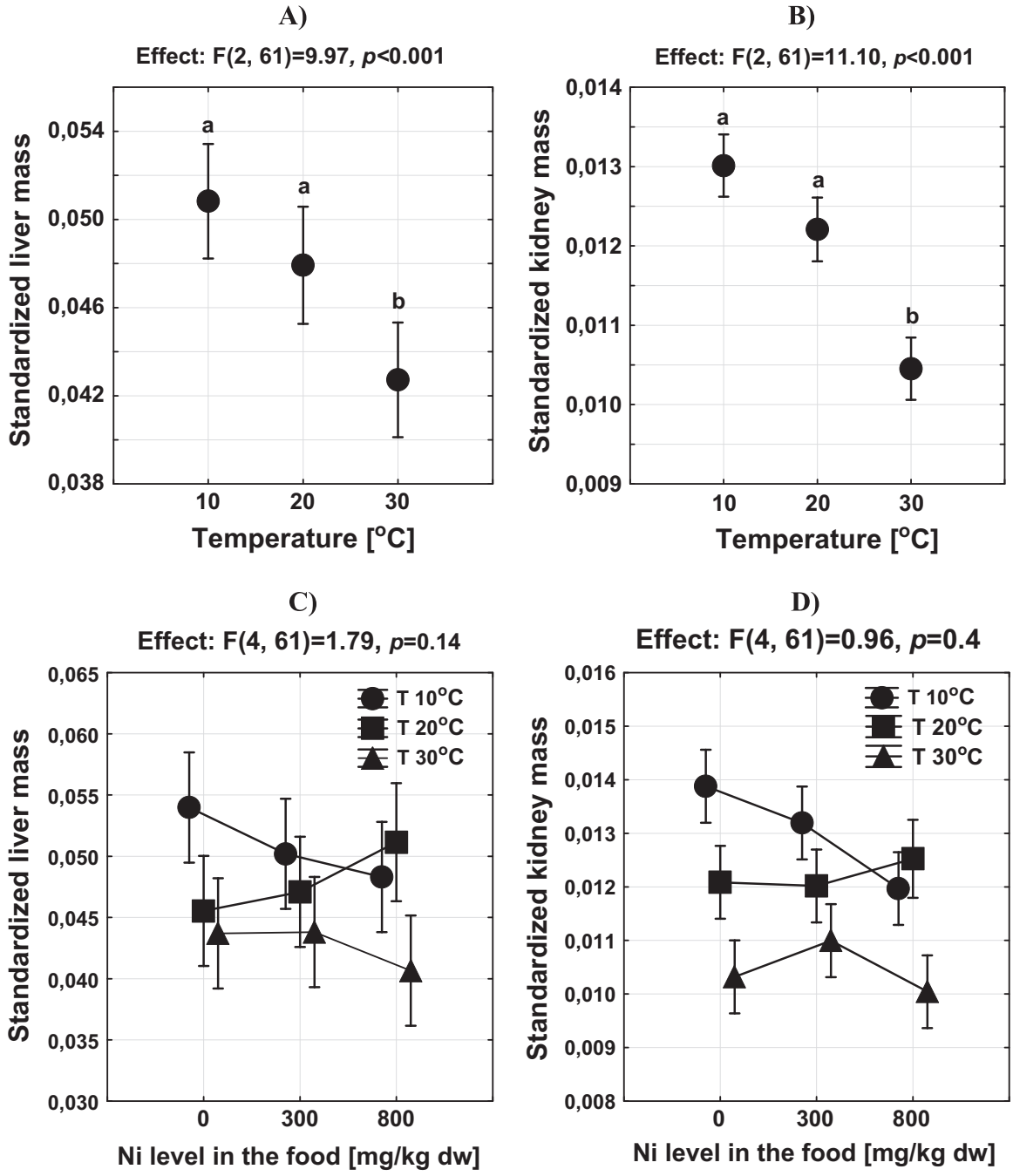

Fig. 5. Effects of temperature on the liver (A) and the kidney (B) masses and the effects of the interaction between temperature (T) and Ni in the food on the liver (C) and the kidney (D) masses. Standardised tissue mass = tissue mass/body mass.

should not be very toxic for the exposed bank voles. Even that some mortality of bank voles would be observed at the highest nickel exposure (Świergosz-Kowalewska et al., 2014) and could also occur in contaminated environments. What is more, as proved by Hou et al. (2011) in their study on rats, the intensive transfer of nickel through the placenta suggests that toxicity may be observed in foetuses and individuals of the next generation. Henderson et al. (2012) found doses of nickel chloride hexahydrate higher than 500 mg/kg given to rats caused intestine necropsy and mortality. Taking into account the fact that the bank vole eats approximately about 3 g of food per day, the highest daily dose of nickel was about 2.5 mg Ni per animal. This was a lower amount of nickel than that given by Henderson et al. (2012), and should not cause such drastic intestine changes.

Our statistical analysis showed that additional stressors in the form of different concentrations of chlorpyrifos and temperature affected nickel levels in the tissues, but that the effects were tissue-dependent. The effects of the factors' interactions were not observed in the gonads, where variation of the results was high (*cf.* SE values at Fig. 1). Nickel concentrations in the liver tissue were influenced by Ni concentrations in the food (so the level of exposure) during the intoxication phase ($p < 0.0001$) (Fig. 2A). Higher Ni food concentrations caused a higher accumulation in the liver. During the elimination phase, the levels of Ni in this tissue were affected by the interaction between Ni in the food and temperature, as well as the day of exposure ($p = 0.042$). However, due to high mortality of animals (Świergosz-Kowalewska et al., 2014), the data for the 63 d of experiment were incomplete and, as

such, the statistical analysis could be affected by that. Comparing the results only for 49 d of exposure, it can be clearly seen that exposure to 10°C resulted in lower Ni accumulation in the liver than at other temperatures. The levels of Ni in the kidney of the bank voles during the intoxication phase were influenced by nickel levels administrated in the food, and the interactions between all the factors (Table 1, Figs. 2B, S1), whereas during the elimination phase only Ni and CPF in the food were significant factors. Because both chemicals were not administrated in this experimental phase, this effect can be explained as a delayed-action.

Forty two day exposure to various stressors outcomes with effects on body mass and organ masses of studied bank voles. Two main factors have an effect on body mass: nickel in the food and temperature. In a review by Holmstrup et al. (2010) most of cited studies showed significant interactions between heat and chemical stress, mainly of a synergistic nature. However, we cannot confirm this in our study. Even the high temperature generally favored high body mass (Fig. 3B), whereas the high 800 mg Ni in the food caused a negative effect (Fig. 3A). The cost of thermoregulation was seen in the case of animals from all the treatments (Fig. 4). After 42 d of exposure to the stressors, animals exposed to the highest concentrations of nickel, 800 mg/kg food, lost significant body mass compared to the other treatments. Two reasons may contribute to this: low food uptake of the contaminated food and/or the toxic effect of the accumulated nickel. We did not observe any avoiding of the contaminated food and temperature positively influenced the animals' body mass, so the toxic effect of nickel was a main reason for the body mass decrease. Such a negative effect on body mass was also observed by Jadhav et al. (2007) after exposure to high doses of metal mixture, also including nickel. The effects of nickel, chlorpyrifos and their mixtures on the body size of *Folsomia candida* was reported by Broerse and van Gestel (2010a, 2010b). They found chlorpyrifos addition to nickel exposure as a positive factor reducing growth retardation. Such an effect was not observed in the case of the studied bank voles.

Similar to the body masses of the studied animals, the masses of the liver and kidney (standardised values) were clearly temperature dependent; at high temperature, the organ masses were lower than at low temperature for all Ni treatments. The effects of low temperature on the liver and kidney masses were especially seen in the case of additional exposure to high Ni concentrations (Fig. 5). It seems that energy expenditure on the detoxification processes during exposure to the two stressors – temperature and nickel – resulted in low organ masses.

5. Conclusions

Even though nickel is not so common in the environment, the rate and level of its accumulation in the tissues of animals and humans, and also factors influencing these processes, should be a special concern of risk assessment. Firstly, because nickel is transferred through the placenta, the rate of Ni absorption and accumulation in the foetus tissues depend on the distribution of Ni in the maternal tissues; the elimination of nickel through the maternal kidney is very quick but not from the foetus's kidney (Hou et al., 2011). Secondly, our study clearly demonstrated that it is not only levels of nickel exposure that affect this metal accumulation in the tissues (in this case, bank voles), but also other stressors, both chemical and physical that are present in the environment, have an impact on nickel distribution and accumulation.

Even though temperature alone had no effects on Ni accumulation in the tissues of the studied bank voles, it may affect this process as an additional stressor. Animals in their natural habitats often face a stressful sub-neutral or high thermal condition, and simultaneously are exposed to various chemicals. For this reason, we believe temperature should be included in all toxicological and ecotoxicological studies, especially into the risk assessment tests.

Acknowledgments

The study was supported by the EU Integrated project No-Miracle (Novel Methods for Integrated Risk Assessment of Cumulative Stressors in Europe [http://nomiracle.jrc.it], contract no. 003956 coordinated by Dr. Hans Lokke at NERI, DK-8600 Silkeborg, Denmark) and the Jagiellonian University (DS-758/2016).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2016.08.015.

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