Chemosphere 149 (2016) 383–390

Contents lists available at ScienceDirect

Chemosphere

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

The effect of chlorpyrifos on thermogenic capacity of bank voles selected for increased aerobic exercise metabolism

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HIGHLIGHTS

- We hypothesized that the rate of metabolism influences susceptibility to pesticides.
- Chlorpyrifos had adverse effects on thermoregulatory performance of bank voles.
- Effects were short-lived and independent of the inherent level of energy metabolism.
- Effects were short-lived and not related to the inherent level of energy metabolism.

ARTICLE INFO

Article history:
Received 22 October 2015
Received in revised form
24 December 2015
Accepted 28 December 2015
Available online 12 February 2016

Handling Editor: David Volz

Keywords:
Bank voles
Organophosphate
Thermogenic capacity
Artificial selection
Chlorpyrifos

ABSTRACT

Agro-chemicals potentially cause adverse effects in non-target organisms. The rate of animal energy metabolism can influence their susceptibility to pesticides by influencing food consumption, biotransformation and elimination rates of toxicants. We used experimental evolution to study the effects of inherent differences in energy metabolism rate and exposure to the organophosphate insecticide, chlorpyrifos (CPF) on thermogenic capacity in a wild rodent, the bank vole (*Myodes* = *Clethrionomys glareolus*). The voles were sampled from four replicate lines selected for high swim-induced aerobic metabolism (A) and four unselected control (C) lines. Thermogenic capacity, measured as the maximum cold-induced rate of oxygen consumption (VO_2cold), was higher in the A − than C lines, and it decreased after continuous exposure to CPF via food or after a single dose administered via oral gavage, but only when measured shortly after exposure. VO_2cold measured 24 h after repeated exposure was not affected. In addition, gavage with a single dose led to decreased food consumption and loss in body mass. Importantly, the adverse effects of CPF did not differ between the selected and control lines.

Therefore, exposure to CPF has adverse effects on thermoregulatory performance and energy balance in this species. The effects are short-lived and their magnitude is not associated with the inherent level of energy metabolism. Even without severe symptoms of poisoning, fitness can be compromised under harsh environmental conditions, such as cold and wet weather.

1. Introduction

The growing extinction of species has raised serious threats to global biodiversity (Butchart et al., 2010; Pimm et al., 1995). Anthropogenic environmental pollutants are suggested as possible drivers of species extinctions (Gibbs et al., 2009; Potts et al., 2010). Organophosphate (OP) insecticides are widely sold and used in agriculture (USEPA, 2007), and levels above maximum residue limits occur in agricultural products (Esturk et al., 2014; Zhao et al., 2014). Rodents inhabit farmlands, so their exposure risk is high although they are non-target organisms. While pesticides may not always kill non-target organisms, they can decrease their physiological performance. OP exposure alters thermoregulation in rodents (Gordon and Granthama, 1999), which may negatively impact the ability to cope with adverse thermal conditions. This can reduce survival and fitness under harsh environmental conditions

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http://dx.doi.org/10.1016/j.chemosphere.2015.12.120
0045-6535/

such as wet and cold weather (DuRant et al., 2007; Moye and Pritsosa, 2010; Piiroinen et al., 2014).

Chlorpyrifos (CPF), a most commonly used OP insecticide (Yan et al., 2012) has been detected in farm products (Rodríguez López et al., 2014; Zhao et al., 2014). Bioactivation of CPF produces a the more neurotoxic CPF-oxon (Buratti, 2003), which inhibits acetylcholinesterase activity, causing repeated firing of cholinergic neurons (Marty et al., 2012). The effects include skeletal muscle tremors, fatigue, weakness, and reduced motor activity (Johnson and Gordon, 1997; Moye and Pritsosa, 2010). Since shivering thermogenesis requires rapid and repeated skeletal muscle activity (Jubrias et al., 2008; Mekjavic et al., 2012), CPF exposure may cause reduced thermogenic capacity and negatively impact fitness in cold environments.

Various biological factors can influence the severity of toxicants' effects (Fritsch et al., 2010). We focused on metabolic rate, a factor which varies widely within species and among species (Careau et al., 2008; Raichlen et al., 2010; Glazier, 2005). For example, there is a 5-fold variation in basal metabolic rate in small rodents of a given mass (Koteja and Weiner, 1993). This variation can influence the severity of toxicants because the rate of metabolism influences food consumption which ultimately causes differential exposure in food. Secondly, differences in the rate of metabolism affect biotransformation and clearance rates of several toxicants (Godin et al., 2006; Rowland et al., 1973; Waser et al., 2010; but see Sims and Steevens, 2008), including CPF (Baas and Kooijman, 2015). Therefore, reactions of wild animals, characterized by very diverse metabolic rates, may differ from those observed in a very limited set of species used in standard toxicological tests.

We tested whether susceptibility of organisms to CPF toxicity differs between lines of the same species with genetically distinct levels of aerobic metabolism. We used bank voles, M*yodes*(=-*Clethronomys*) *glareorus,* from four A-lines, selected for high swim-induced aerobic metabolism (VO_2swim), and four unselected, control C-lines (Sadowska et al., 2008). Voles from the A lines had higher VO_2swim than voles from the C lines and had an increased basal metabolic rate (Sadowska et al., 2015). Three separate experiments detailed under sections 2.2, 2.3 and 2.4 were performed to test the effects of metabolic rate and CPF exposure on thermogenic capacity.

We predicted that CPF exposure would decrease thermogenic capacity but contradictory hypotheses about the rate of metabolism can be proposed. At a high rate of metabolism, a greater decrease in thermogenic capacity is possible due to rapid generation of CPF-oxon and increased effective exposure through increased consumption of contaminated food. Alternatively, a reduced effect is possible due to a faster biotransformation and elimination of the toxicant from the body.

2. Materials and methods

2.1. The artificially selected bank vole as a model organism

The bank vole (*Myodes* = *Clethrionomys glareolus*) is a common European rodent, widely used as a model in a variety of ecotoxicological research (Damek-Poprawa and Sawicka-Kapusta, 2004; Gdula-Argasińska et al., 2004; Savva et al., 2002; Swiergosz et al., 1998). The study used bank voles from an artificial selection experiment with four randomly bred control lines (C) and four lines selected for maximum swim-induced aerobic metabolism (A) (Sadowska et al., 2008). Details about the colony and selection experiment protocols can be found in Sadowska et al. (2008, 2015). The animals were sampled from generations 11, 12 and 18, in which the maximum swim-induced aerobic metabolism in A lines was about 50% higher than C lines. A complete transcriptome analysis of hearts and livers showed that differences between the selected and control lines in gene expression levels were more profound in the liver, suggesting that the selection acted on whole metabolism and not just exercise-related metabolism (Konczal et al., 2015).

The voles were maintained in standard plastic mouse cages (mostly opaque, polypropylene) with sawdust bedding, at a constant temperature (20 ± 1 °C) and photoperiod (16 h:8 h light:dark: light phase starting at 3:00 am). Air humidity in the animal rooms varied depending on outside ambient conditions.

Water and food (rodent chow: 24% protein, 3% fat, 4% fiber: Labofeed H, Kcynia, Poland) were provided ad libitum. All cages were visually inspected for presence of food, water and dead animals every day. All procedures performed on animals were approved by the Local Bioethical Committee in Kraków, Poland (Decision No. 99/2006, 21/2010, 22/2010 and 61/2011).

2.2. Experiment 1: exposure in contaminated food

Animals were given either a diet contaminated with CPF (CPF diet) or an uncontaminated diet (Control diet). Food pellets for both diets were prepared two days before start of the experiment. To prepare the CPF diet, a dough containing 1 kg of powdered rodent feed (Labofeed H, Kcynia, Poland) was mixed with a solution of 104 μl of Dursban (480 gCHP/L, DowAgrosciences) in 1250 ml of lukewarm tap water. This preparation produced contaminated food pellets containing 50 mg of CPF/kg of feed. The Control diet was prepared in a similar way, but Dursban was replaced by an extra 104 μl of water. CPF food pellets were molded by running portions of this dough through a meat mincer. Pellets were then dried under a vacuum in a laboratory hood for 40 h. Dried CPF diet and Control diet pellets were stored in separate sealed buckets at 20 °C.

Male and female bank voles (n = 160) from A and C lines of generation 11, were used. The animals were assigned to two CPF treatment groups (CPF diet vs Control diet), balanced with respect to Linetype (A vs C lines), replicate lines within the selection groups, and sex. Water and food (15–20 g) were provided ad libitum for two days. Thus, unlike in the next two experiments, the CPF diet group was continuously exposed to CPF for the two days. After this time, thermogenic capacity (VO_2cold) was measured following the protocol described in section 2.5. Body mass was recorded at the start and end of feeding trials. Change in body mass was calculated by subtracting final body mass from the starting body mass. Data from five voles were deleted as outliers (resulting from errors in measuring food consumption).

All uneaten food from each individual was collected and dried to constant mass for two days at 65 °C. Samples were then weighed to obtain the dry mass content. Dry mass content in fresh food (proportion) was calculated from calibration food samples, which were dried in the same way as the uneaten food. Total food consumption (FC, g) per individual over the experimental period (two days) was calculated as:

$$\mathrm{FC} = (\text{mass of food given} \times \text{dry mass content}) - (\text{mass of dry uneaten food}).$$

Total consumption of CPF was calculated as food consumption multiplied by the concentration of CPF (50 mgCPF/kg). The dosage consumed was defined as daily CPF consumption (total consumption divided by two) per gram of the initial body mass.

2.3. Experiment 2: repeated administration of controlled dosages

Males and females (n = 256) from A and C lines of generation 12 were used. Animals were assigned to three CPF Treatment groups (CPF300 – 300 μg/d, CPF60 – 60 μg/d, and CPF0 – rapeseed oil