



Effect of ethofumesate herbicide on energy metabolism in roach (*Rutilus rutilus*)

Virginie Maes, Aurélie Vettier, Odile Dedourge-Geffard, Alain Geffard,
Séverine Paris-Palacios, Stéphane Betouille, Elise David

► To cite this version:

Virginie Maes, Aurélie Vettier, Odile Dedourge-Geffard, Alain Geffard, Séverine Paris-Palacios, et al.. Effect of ethofumesate herbicide on energy metabolism in roach (*Rutilus rutilus*). Journal of Xenobiotics, 2014, 4 (2), pp.4901. 10.4081/xeno.2014.4901 . hal-04259062

HAL Id: hal-04259062

<https://hal.univ-reims.fr/hal-04259062>

Submitted on 25 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Effect of ethofumesate herbicide on energy metabolism in roach (*Rutilus rutilus*)

V. Maes, A. Vettier, O. Dedourge-Geffard, A. Geffard, S. Paris-Palacios, S. Betouille, E. David

Université de Reims Champagne-Ardenne, UMR-I 02, Stress Environnementaux et BIOSurveillance des milieux aquatiques (SEBIO), FR CNRS 3417 Condorcet, UFR Sciences Exactes et Naturelles, Reims, France

Introduction

Ethofumesate is a benzofuran herbicide commonly used to control weeds of sugar beet. Its mechanism of action in plants is to inhibit the synthesis of very long chain fatty acids (>C18). Concentration of ethofumesate in surface freshwater vary between 0.5 and 3 $\mu\text{g.L}^{-1}$ ^{1,2} but maximal concentration was found in Germany at 51.1 $\mu\text{g.L}^{-1}$.³ Although herbicides are designed to eliminate unwanted plants, they could present toxicity against non-target organisms. Thus, herbicides are considered dangerous at low concentrations to both aquatic invertebrates and vertebrates.^{4,5} Therefore, there is an increasing need to understand the toxicity of such chemicals on non-target aquatic organisms. Studying the energy metabolism constitutes an appropriate approach to detect physiological disturbances of organisms linked to their exposure to pollutants. Indeed animal survival depends on the availability of energy necessary to ensure physiological functions as maintenance, growth and reproduction.

The roach (*Rutilus rutilus*) is a cyprinid species found throughout Europe. Roach can be easily identified and caught and is found in large populations. It is commonly or often used for biological biomonitoring studies of aquatic environments.⁶ Because of its robustness, it can develop in polluted environments and thus constitute a good bioindicator.⁷ The aim of this study was to determine the effect of ethofumesate on energy metabolism in juvenile roach at different (biochemical, molecular and cellular) levels. Additionally, two temperatures were tested (10 and 17°C) to assess potential effects of this environmental parameter on energy metabolism responses to chemicals. Among biological processes involved in cellular energy synthesis, we focused on glycolysis and respiratory chain pathways.

Materials and Methods

Exposure conditions

Juvenile roach (*Rutilus rutilus*) were purchased from a commercial pond farm located in Champagne-Ardenne region (France). After 10 days of acclimatization, fish were exposed to 0; 0.5; 5 or 50 $\mu\text{g.L}^{-1}$ of ethofumesate for seven days, at 10°C and at 17°C. During exposure, water was replaced every day to maintain ethofumesate concentration. Ethofumesate concentrations were checked before and after each water replacement. All along acclimatization and exposure, fish were fed *ad libitum* every two days with mud worms and photoperiod was kept constant (LD 12:12). No mortality was observed during acclimatization and exposure. Nine fish were sacrificed at the beginning of each experiment (T_0) then nine fish per condition were sampled after 1 (T_1) and 7 (T_7) days. White muscle was sampled, flash frozen in liquid nitrogen and kept at -80°C until biochemical and molecular analyses. For cellular analyses, muscle was fixed with glutaraldehyde.

Analyses

The glycolysis pathway was studied at two regulation levels in white muscle of juvenile roach. First, a biochemical approach was carried out with the glycolytic fluxes measurement. This biochemical spectrophotometric method allows measuring the aerobic (J_A) and anaerobic (J_B) capacities of the first steps of glycolysis on white muscle homogenate.⁸ Glycolytic activity was assessed from the NADH decrease at 340 nm. The reaction was triggered by adding glucose. The system reached a steady state called J_A , representative of the aerobic flux of glycolysis, stimulated by free glucose in aerobic conditions. After 5 min of steady state, glucose-6-phosphate (G6P) was added to the mixture, and the system reached a new steady state called J_B . J_B represents the anaerobic flux of glycolysis, stimulated by glycogen in anaerobic conditions. Secondly, a molecular approach was studied by measurement of the relative expression of 4 genes encoding for glycolysis enzymes (Hexokinase, HK; Phosphofructokinase, PFK; Glyceraldehyde 3-phosphate dehydrogenase, G3PDH and Pyruvate kinase, PK; Table 1) by real-time quantitative PCR according to Livak and Schmittgen.⁹ Actin and Ribosomal protein L8 (Rpl8) genes were used as housekeeping genes (Table 1).

The respiratory chain pathway was studied at three regulation levels. First, a biochemical approach was carried out with the activity of electron transports system (ETS) according to De Coen and Janssen.¹⁰ This method lies on the saturation of electron flux through mitochondrial membrane by adding high levels of

Correspondence: Virginie Maes, Université de Reims Champagne-Ardenne, UMR-I 02, Stress Environnementaux et BIOSurveillance des milieux aquatiques (SEBIO), FR CNRS 3417 Condorcet, UFR Sciences Exactes et Naturelles, BP 1039, 51687 Reims cedex 2, France.
E-mail: virginie.maes@univ-reims.fr

Key words: ethofumesate herbicide, *Rutilus rutilus*.

Conference presentation: ECOBIM meeting, 2014 May, Brest, France.

This work is licensed under a Creative Commons Attribution NonCommercial 3.0 License (CC BY-NC 3.0).

©Copyright V. Maes et al., 2014
Licensee PAGEPress, Italy
Journal of Xenobiotics 2014; 4:4901
doi:10.4081/xeno.2014.4901

natural substrates (NADH and NADPH). This activity was measured spectrophotometrically and following the formazan production during 6 min at 490 nm. Secondly, a molecular approach was studied by the measurement of the relative expression of cytochrome c oxidase subunit 1 (CCOx1; Table 1). Thirdly, a cellular approach was performed by the observation of mitochondria ultrastructure. Muscle was fixed with glutaraldehyde (3%), included in resin and cut at 50 nm. Ultrathin sections were stained with lead citrate and examined with a transmission electron microscope.

Statistical analysis

Statistical analysis was performed using Minitab 16 software. As all parameters were non-normally distributed (Kolmogorov-Smirnov test), non-parametric Kruskall-Wallis and Mann-Whitney U tests were used. Results are expressed as mean±S.E.M, excepted for molecular analysis where a box plot was used.

Results and Discussion

Focusing on glycolysis pathway, no significant difference in PK, PFK and G3PDH genes expression was observed between conditions or times of exposure. Expression of HK gene tended to decrease at T_1 when ethofumesate concentrations increased in fish exposed at 10°C, with a significant decrease in roach exposed to 50 $\mu\text{g.L}^{-1}$ of ethofumesate (Figure 1). This trend was not observed after seven days of exposure, excepted in roach exposed to 50 $\mu\text{g.L}^{-1}$ of ethofumesate who showed a lower relative gene expression of HK than control. As HK is the only aerobic enzyme of the glycolysis pathway, its activity in vertebrate muscle is

directly related to the respiratory capacity.¹¹ An under-expression of HK could involve a disturbance in aerobic energy production. In our study, no significant difference in aerobic or in anaerobic flux was observed at enzymatic activity level, suggesting compensatory mechanisms between the two regulation levels when fish were exposed to ethofumesate at 10°C. It is well known that glycolytic flux is essentially regulated at enzymatic activity level, in particular the one of HK, PFK and PK which corresponds to the 3 allosteric enzymes.^{12,13} Nevertheless, further investigations are needed to understand the compensatory mechanisms involved in the present study.

When roach were exposed to ethofumesate at 17°C, no significant difference was observed with control for all glycolysis genes expression, but a decrease in the aerobic flux (J_A) and an increase in the anaerobic flux (J_B) were observed in contaminated fish at both times of exposure as revealed by J_B/J_A ratio (Figure 2). The increase in anaerobic flux is no significant due to the high variability between individuals. These results suggest that ethofumesate can act directly on glycolytic enzymes and/or their substrates to disturb the glycolytic flux. Using the anaerobic metabolism involves consequences in terms of energy budget. Indeed, the anaerobic pathway is less effective than aerobic one for producing cellular energy (2 ATP molecules vs 38). Consequently, an increase in anaerobic metabolism may result in an extensive use of energy reserves that can in return affect physiology and survival. The increase in anaerobic flux observed in the present study, could aim to compensate the decrease in aerobic flux to allow a maintained cellular energy production, despite a lowest efficiency. The lower ATP production linked to the activation of the anaerobic pathway may

lead to a reduction of energy availability for physiological functions of organism (growth, reproduction, immunity...). Our results suggest that under ethofumesate exposure, energy is produced essentially to ensure fish survival at the expense of others process.

Focusing on respiratory chain pathway, when roach were exposed to ethofumesate at 10°C, no significant difference was observed with control, whether on ETS activity, or on CCOX1 expression. On the other hand, mitochondria disturbances were observed (Figure 3). Indeed, alterations or destructions of mitochondrial structures were observed in the outer membrane, the cristae and in the general mitochondria shape. The cellular regulation level is generally expected to be affected by chemicals after the molecular or biochemical one. Generally, mitochondria disturbances are

correlated to respiratory chain and/or oxidative phosphorylation impairments and ROS production.^{14,15} In the present study, ethofumesate may act on the oxidative phosphorylation and on the ATP synthase activity.

When roach were exposed to ethofumesate at 17°C, no significant difference was observed for CCOX1 expression between control and contaminated fish and times of exposure. However, a decrease in ETS activity was observed at T₁, in roach exposed to ethofumesate compared to control, significant in roach exposed to 5 µg.L⁻¹ of ethofumesate (Figure 4). This decrease was not observed after 7 days. These results suggest that ethofumesate can act directly on respiratory chain enzymes. ETS represents a valid alternative measure to whole animal respiration.¹¹ It's assumed that ETS activity is an overestimation of the maxi-

Table 1. Primer sequences used for quantitative real-time polymerase chain reaction.

Gene	NCBI Accession n°	Sense	5'-3' Sequence
HK	HF544501.1	For Rev	AGTTGCTGCAGAATGGACCT TGCAGAACATCTTGATTGAC
PFK	HF544502.1	For Rev	TGCTTCAGCAAGATTTGGA TCCGCACTAAGGAGGGTAGA
C3PDH	HF544500.1	For Rev	ACGCTATCACAGCCACACAG AGGCCATGCCAGTAATCTTG
PK	HF544503.1	For Rev	ATTGGTCGTTGCAACAGGAT TCCCCACTCAGCATGATACA
CCOX1	HQ600768.1	For Rev	GGGTCACTTTAGGCATGA TTCGTGGAAATGCTATGTCA
Rpl8	FJ769335	For Rev	ATCCCAGACCAAGAAATCCAGAG CCAGCAACAACCCAACAACAG
Actin	DQ061948.1	For Rev	GCTGGAAGCAGCAGGTTATC CACACCATCCACACATCCAT

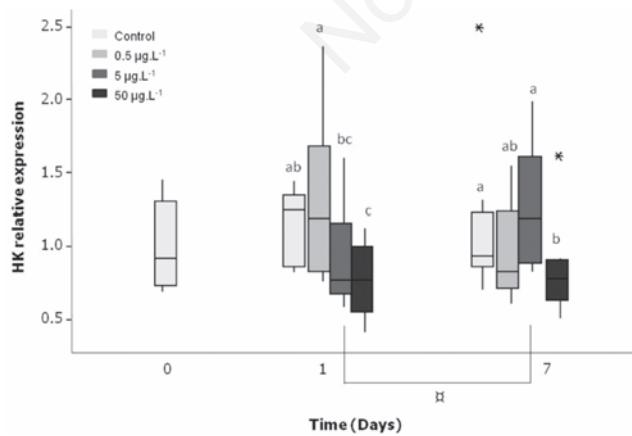


Figure 1. Relative gene expression of hexokinase during ethofumesate exposure at 10°C in white muscle of roach. Different letters indicate significant differences for a same time ($P<0.05$). *Corresponds to outliers. Bars with □ indicate significant differences for a same concentration.

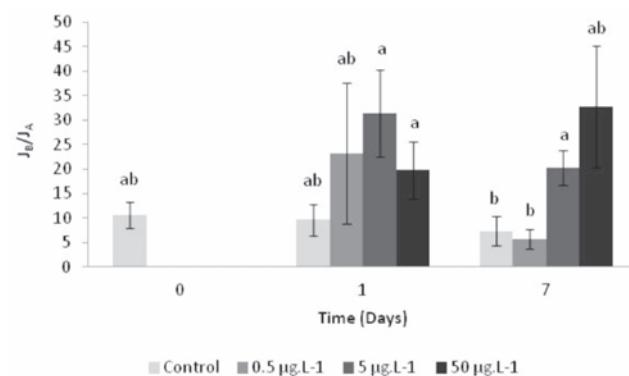


Figure 2. JB/JA ratio during ethofumesate exposure at 17°C in white muscle of roach. Different letters indicate significant differences ($P<0.05$).

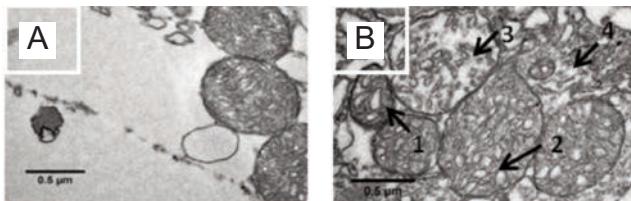


Figure 3. Ultrastructure of muscular mitochondria in roach exposed to $50 \mu\text{g}\cdot\text{L}^{-1}$ after one day of ethofumesate exposure at 10°C . A) Electron micrograph from control roach muscle showing round regular mitochondria. B) Electron micrograph from contaminated roach muscle showing disrupted outer mitochondrial membrane (1), dilated cristae (2), destroyed cristae and absent matrix (3), and mitochondria entirely destroyed (4).

mal cellular respiration. Maintaining electron transport activity at a significant level is essential since mitochondrial electron transport chain supplies over 95% of the total ATP requirement.¹⁶ Then the return to the control level after 7 days could correspond to a compensative way to prevent an excessive loss of cellular energy, especially ATP.

The mechanism of action of ethofumesate on cellular energy metabolism in animals remains unknown, but if this molecule affects the lipids synthesis in animals as it does in plants, this could have such consequences on respiratory chain pathway. Indeed, lipids provide substrates of aerobic metabolism (Krebs cycle and respiratory chain pathways) through β -oxidation. If the lipids synthesis is affected by ethofumesate, the aerobic metabolism could potentially be affected, with a decrease in electron transfer through respiratory chain pathway, and thus could potentially activate the anaerobic metabolism.

Moreover, mitochondria disturbances were observed (data not shown) with same effects as mentioned above. However, these effects were observed in contaminated fish but also in control. We can hypothesize that these cellular alterations were linked to a temperature effect, but such disturbances were not observed with other parameters. However, Van Dijk *et al.*¹⁷ showed that, when fish were fed *ad libitum*, preferential temperature for roach growth was 26°C and that the optimal temperature range for growth was $20\text{--}27^\circ\text{C}$. However, mitochondria disturbances due to hyperthermia are well-documented,^{18,19} showing dilated cristae, swollen mitochondria, and modified shape.

In conclusion, this study revealed disturbances on muscular energy metabolism after ethofumesate exposure on juvenile roach, especially in glycolysis pathway. Moreover, at 17°C an effect on respiratory chain was observed. Ethofumesate induced more effects

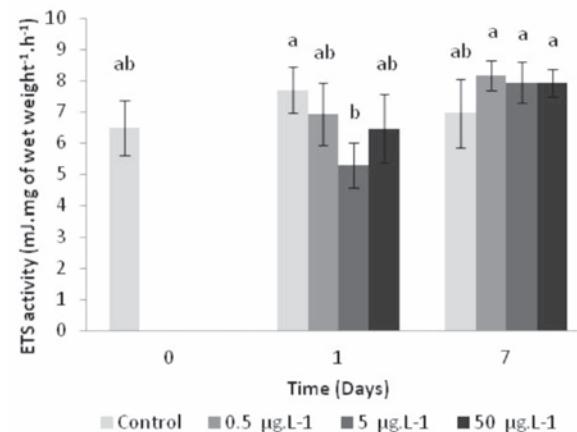


Figure 4. Electron transport system activity during ethofumesate 17°C exposure in white muscle of roach. Different letters indicate significant differences ($P<0.05$).

at 17 than 10°C , what seems consistent, as energy metabolism is known to be more active with increasing temperature.²⁰ This study underlines the importance of temperature as confounding factor. Thus, environmental temperature is an important parameter to take into account when studying such effects of pesticides.

References

- Berenzen N, Kumke T, Schulz HK, Schulz R. Macroinvertebrate community structure in agricultural streams: impact of runoff-related pesticide contamination. *Ecotox Environ Safe* 2005;60:37-46.
- Herrero-Hernández E, Andrades MS, Álvarez-Martín A, Pose-Juan E, Rodríguez-Cruz MS, Sánchez-Martín MJ. Occurrence of pesticides and some of their degradation products in waters in a Spanish wine region. *J. Hydrol* 2013;486:234-45.
- Neumann M, Liess M, Schulz R. A qualitative sampling method for monitoring water quality in temporary channels or point sources and its application to pesticide contamination. *Chemosphere* 2003; 51:509-13.
- Vidal T, Abrantes N, Gonçalves AMM, Gonçalves F. Acute and chronic toxicity of Betanal® expert and its active ingredients on nontarget aquatic organisms from different trophic levels. *Environ Toxicol* 2010;27:537-48.
- Moore MT, Pierce JR, Milam CD, Farris JL, Winchester EL. Responses of non-target aquatic organisms to aqueous propanil exposure. *Bull Environ Contam Toxicol* 1998;61:169-74.
- Chovanec A, Hofer R, Schiener F. Chapter 18: Fish as bioindicators. In: Markert BA, Breure AM, Zechmeister HG, eds. *Trace metals and other contaminants in the environment. Vol. 6: Bioindicators & Biomonitoring - Principles, Concepts and Applications*. Dordrecht: Elsevier; 2003. pp 639-676.
- Bruslé J, Quignard JP. *Biologie des poissons d'eau douce européens*. Paris: Edition TEC&DOC; 2001. pp 199-211.
- Sébert P, Peragon J, Barroso JB, Simon B, Meléndez-Hevia E. High hydrostatic pressure (101 ATA) changes the metabolic design of yellow freshwater eel muscle. *Comp Biochem Physiol Part B* 1998;121:195-200.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_t}$ method. *Methods* 2001;25:402-8.
- De Coen WM, Janssen CR. The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular Energy Allocation: a new methodology to assess the energy budget of toxicant-stressed *Daphnia* populations. *J Aquat Ecosyst Stress Recovery* 1997;6:43-55.
- Burleigh JG, Schimke RT. On the activities of some enzymes concerned with glycogenolysis in extracts of rabbit skeletal muscles. *Biochem Biophys Res Commun* 1968;31:831-6.
- Uyeda K, Racker E. Regulatory mechanisms in carbohydrate metabolism. VII. Hexokinase and Phosphofructokinase. *J Biol Chem* 1965;240:4682-8.
- Purich DL, Fromm HJ. Studies on factors influencing enzyme responses to adenylylate energy charge. *J Biol Chem* 1972;247:249-55.
- Lei W, Wang L, Liu D, Xu T, Luo J. Histopathological and biochemical alterations of the heart induced by acute cadmium exposure in the freshwater crab

- Sinopotamon yangtsekiense. Chemosphere 2011;84:689-94.
15. Al Kaddissi S, Legeay A, Elia AC, Gonzalez P, Camilleri V, Gilbin R, et al. Effects of uranium on crayfish *Procambarus clarkii* mitochondria and antioxidants responses after chronic exposure: What have we learned? Ecotox Environ Safe 2012;78:218-24.
16. Ereci ska M, Wilson DF. Regulation of cellular energy metabolism. J Membrane Biol 1987;70:1-14.
17. Van Dijk PLM, Staaks G, Hardewig I. The effect of fasting and refeeding on temperature preference, activity and growth of roach, *Rutilus rutilus*. Oecologia 2002;130:496-504.
18. Arancia G, Crateri Trovalusci P, Mariutti G, Mondovi B. Ultrastructural changes induced by hyperthermia in Chinese hamster V79 fibroblasts. Int J Hyperthermia 1989;5:341-50.
19. Welch WJ, Suhan JP. Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heatshock treatment. J Cell Biol 1985;101:1198-211.
20. Lemieux H. Effets de la température sur le métabolisme mitochondrial cardiaque. Thesis, Rimouski, Canada: Québec University; 2007.