Risk of combined exposure of birds to cyanobacterial biomass containing microcystins, acetylcholinesterase inhibitor and anticoagulant

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Abstract

OBJECTIVES: The objective of this study was to examine the hypothesis that a combination of cyanobacterial biomass containing microcystins, acetylcholinesterase inhibitor and anticoagulant can enhance avian toxic effects produced by single exposures only.

METHODS: A total of 48 two-month-old Japanese quails (*Coturnix coturnix japonica*) with average body weight of 160 g were randomly divided into 8 experimental groups of six birds and sex ratio of 1:1. Experimental groups of control Japanese quails (C) and birds exposed to single and combined sub-lethal doses of paraoxon (P), bromadiolone (B), and microcystins in cyanobacterial biomass (M) included: C, P, P+B, B, B+M, P+M, M, and P+B+M. During the 10-day exposure birds in the respective groups received biomass containing 61.62 μg microcystins daily (i.e. 26.54 μg MC-RR, 7.62 μg MC-YR and 27.39 μg MC-LR), two 250 μg/kg doses of paraoxon, and two 500 mg/kg doses of bromadiolone. Group responses were compared using standard plasma biochemistry and antioxidant/oxidative stress parameters in tissues.

RESULTS: While single and double combinations of toxicants induced responses in individual biochemical parameters measured and evaluated using univariate statistical analysis, those in the triple exposure were most extensive. The principal component analysis of antioxidant/oxidative stress parameters (glutathione reductase, lipid peroxidation, and ferric reducing antioxidant power) in tissues (liver, kidney, heart, brain, lungs, gonads, and pectoralis major muscle) clearly separated the triple group (P+B+M) from all single and double exposure groups and the control and indicated thus marked joint effects in the overall pattern of antioxidant/

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oxidative stress responses of this group. The separation was driven by the modification of the ferric reducing antioxidant power levels in heart and brain and the cardiac lipid peroxidation level, in particular.
CONCLUSIONS: This experiment contributes to the understanding of the pathogenic mechanisms of combined sub-lethal exposure to natural toxins and agrochemicals and may be used for risk assessment of environmental pollution in birds.

Abbreviations:

ALT	- alaninaminotransferase
ANOVA	- analysis of variance
C	- controls
B	- bromadiolone-exposed quails
FRAP	- ferric reducing antioxidant power
GR	- glutathion reductase
HDL	- high-density lipoprotein
LSD test	- the least significant difference test
MC	- microcystin
MC-RR	- microcystin RR
MC-YR	- microcystin YR
MC-LR	- microcystin LR
NADPH	- nicotinamide adenine dinucleotide phosphate
OECD	- Organisation for Economic Co-operation and Development
P	- paraoxon-exposed quails
P+B	- paraoxon-and-bromadiolone-exposed quails
P+B+M	- paraoxon-, bromadiolone-and-microcystins-exposed quails
PCA	- principal component analysis
TBARS	- thiobarbituric acid reactive species

INTRODUCTION

Birds can constantly be exposed to different chemicals and multiple stressors in the environment, both of natural and anthropogenic origin (Carson 1962; Fry 1995; Pikula *et al.* 2010; Rattner 2009). One possible scenario is that of combined exposure to cyanotoxins and pesticides. While mortalities and even mass deaths have been attributed to natural blooms of toxigenic cyanobacteria (Alonso-Andicoberry *et al.* 2002; Damkova *et al.* 2009; Damkova *et al.* 2011; Krienitz *et al.* 2002; Lopez-Rodas *et al.* 2008; Skocovska *et al.* 2007), birds may also be exposed to cholinesterase-inhibiting pesticides such as organophosphorus and carbamate compounds to control insects (Strum *et al.* 2010) or rodenticidal anticoagulants (Sanchez-Barbudo *et al.* 2012) in agricultural habitats.

In contrast with mortalities, sub-lethal effects or exposure to low levels of toxicant mixtures are less recognised and documented (Pikula *et al.* 2010), although chronic and indirect effects may be even more hazardous for animal populations on a long-term basis (Berny 2007). Apart from their primary modes of action, toxic substances can elicit clinical responses in birds that may include oxidative stress as an unspecific biochemical process involved in the adverse reaction to many stressors. While Paskova *et al.* (2008) reported induction of oxidative stress along with accumulation of microcystins in Japanese quails, reports on avian poisonings by anticholinesterases and anticoagulants deal mainly with the laboratory diagnostics part of the problem (Beklova *et al.* 2007; Guitart *et al.* 2010; Krizkova *et al.* 2007).

The question of the impact of multiple exposures has only sporadically been examined experimentally in birds. The objective of this study, therefore, was to test the hypothesis that exposure to cyanobacteria containing microcystins, cholinesterase inhibitors and anticoagulants can interact and result in more pronounced toxic effects than single exposures only. For this purpose we compared the effects of cyanobacterial biomass, paraoxon and bromadiolone in Japanese quails in single, double and triple exposures and evaluated the clinical signs, mortality, standard plasma biochemistry and levels of antioxidant and oxidative damage parameters in selected organs.

MATERIAL AND METHODS

Experimental animals

Two-month-old Japanese quails (*Coturnix coturnix japonica*) were used as a model avian species. Healthy animals in excellent nutritional state (average body weight of 160 g) were selected for the experiment performed in compliance with the laws for the protection of animals against cruelty and approved by the Ethical Committee of the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic.

Experimental design

The experiment was conducted according to the OECD guideline 205 modified for our experimental conditions. Birds were allowed to acclimatise for 10 days prior to 10 days of testing. A total of 48 specimens were randomly divided into 8 experimental groups of six birds with the sex ratio of 1:1 in each group. Following eight experimental groups of control Japanese quails (C) and birds exposed to single and combined doses of paraoxon (P), bromadiolone (B), and microcystins in cyanobacterial biomass (M) were formed: C, P, P+B, B, B+M, P+M, M, and P+B+M.

Experimental substances

The cyanobacterial biomass with content of microcystins (MCs) used in the present study was collected using a plankton net (25 μm) from the Musovska reservoir (Czech Republic) in August 2007. The biomass dominated by *Microcystis* sp. included 90% *M. aeruginosa* and 10% *M. ichthyoblabe*. It was lyophilised and mixed into the diet. The lyophilisation was conducted with Christ Gamma 1-16 LSC Freeze dryer (Osterode am Harz, Germany). A vertical feed mixer HV 100 (Pharmix, Kromeriz, Czech Republic) providing a guarantee of high-grade mixture homogeneity independent of admission degree and enabling payload volumes from 50 to 5000 L was used to prepare the experimental feeding mixture. The homogeneous distribution and stability of MCs in the diet was verified by labora-

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tory analysis. The daily dose of the biomass containing 61.62 μg MCs per bird included 26.54 μg MC-RR, 7.62 μg MC-YR and 27.39 μg MC-LR. The contents of MCs in the biomass and feed were analysed using high performance liquid chromatography with diode array detection (HPLC-DAD; Agilent 1100 Series, Agilent Technologies, Japan) on a Supelcosil ABZ + Plus column, 150 x 4.6mm, 5 μm, according to the method by Babica *et al.* (2006). Birds from all groups were provided with drinking water *ad libitum*. Non-cyanobacterial-biomass-exposed birds were allowed free access to the feeding mixture without addition of blue-green algae. Two sub-lethal doses of paraoxon (250 μg/kg; Paraoxon-ethyl, analytical standard, Fluka Analytical, Sigma-Aldrich, St. Louis, USA; 0.1ml of propylenglycol was used for dilution of paraoxon, Propylenglycolum, Tamda, a.s., Olomouc, Czech Republic) were gavage applied to each bird in the single and combined paraoxon-treated groups on day 1 and 5 of the experimental period. The total dose of paraoxon thus amounted to 500 μg/kg. Likewise, the anticoagulant bromadiolone purchased from Sigma Aldrich (Steinheim, Germany) was administered by a crop probe in two doses of 500 mg/kg on days 1 and 5 of the experiment to birds from groups P+B, B, B+M, and P+B+M. Therefore, the total dose of bromadiolone amounted to 1 000 mg/kg in each bird. The sublethal doses of paraoxon and bromadiolone in this study were derived as approximately one fourth to one half of the LD_{50}.

Data and samples collected

Birds were examined every 6 hours for developing signs of toxicity, blood sampled and euthanized after ten days of exposure. On necropsy, whole organs including liver, both kidneys, heart, brain, lungs, and gonads (testes or ovaries) were dissected, weighed and stored deep frozen at –80 °C for later analyses.

Biochemistry

Blood (2 ml) was collected from the right jugular vein using a 2 ml heparinised syringe (B.Brown Injekt®, Germany) and a 25-gauge needle (0.5×25 mm, Terumo Europe, Belgium), and was processed as previously described (Vitula *et al.* 2011). Whole blood samples were analysed using an automated analyser (SPOTCHEM™ EZ SP-4430, ARKRAY, Japan) for calcium (mmol/l), phosphorus (mmol/l), magnesium (mmol/l), glucose (mmol/l), total cholesterol (mmol/l), high-density lipoprotein cholesterol (mmol/l), triglycerids (mmol/l), total protein (g/l), uric acid (mmol/l), amylase (μkat/l), aspartate aminotransferase (μkat/l), alkaline phosphatase (μkat/l), creatine kinase (μkat/l), lactate dehydrogenase (μkat/l), alaninaminotransferase (μkat/l), and bilirubin (μmol/l).

Antioxidant parameters including glutathione reductase, lipid peroxidation, and total antioxidant capacity were measured in tissue samples as described previously (Benzie & Strain 1996; Paskova *et al.* 2008, Pohanka *et al.* 2009). Activity of glutathione reductase (GR) was determined from the rate of NADPH oxidation. The level of lipid peroxidation in avian tissues was assessed as total thiobarbituric acid reactive species (TBARS). The total antioxidant capacity was measured using the ferric reducing antioxidant power assay (FRAP).

Statistical analysis

Statistica for Windows® 10 (StatSoft, Inc., Tulsa, OK, USA) was used to compare different experimental groups using procedures such as one-way analysis of variance (ANOVA), post-hoc analysis of means by the LSD test, Levene's method to test for the homogeneity of variances, log-transformation of non-homogenous parameters prior to analysis and comparison with the non-parametric Kruskal-Wallis test. The levels of significance used were either $p<0.05$ or $p<0.01$. Multivariate analysis of antioxidant/oxidative damage parameters data (i.e., glutathione reductase, lipid peroxidation, and ferric reducing antioxidant power in tissues of liver, kidney, heart, brain, lungs, gonads, and pectoralis major muscle) was performed using principal component analysis.

RESULTS

Mortality and clinical signs

No symptoms of intoxication were observed and all experimental birds survived the 10-day period of acute exposure. Food intake, activity and alertness were not decreased. There was no macroscopic evidence of bleeding in bromadiolone-exposed groups (B, P+B, B+M, and P+B+M). Necropsy findings were negligible.

Blood biochemical profiles and tissue antioxidants

Biochemical responses of birds from all experimental groups provided a great number of differences when compared with the control. Table 1 shows significant responses of tested blood biochemical parameters (increases only recognised), while Table 2 documents both increases and decreases of tissue antioxidants and lipid peroxidation. While the lowest value of total cholesterol was measured in the control group (4.24±1.09 mmol/l), the highest value was found in the B+M group (6.11±0,95 mmol/l). As shown in comparison with controls, all increases in total cholesterol were statistically significant, apart from the P group. Increases in high-density lipoprotein cholesterol were observed in groups P+B, B+M, P+M and M.

The ferric reducing antioxidant power (FRAP), a total antioxidant capacity parameter, amounted to the highest level of 4.33±0.54 μmol/g in the pulmonary tissue of the triple exposed group (P+B+M). A significant increase in pulmonary FRAP value was observed also in group M. A marked increase in the cardiac FRAP was demonstrated in the P+B+M group only. Likewise, the highest lipid peroxidation (TBARS assay)

Tab. 1. Responses of tested plasma biochemical parameters of birds exposed to single and combined doses of paraoxon (P), bromadiolone (B), and microcystins in cyanobacterial biomass (M) compared against control Japanese quails (C).

	Total chol.	HDL chol.	Mg	P	ALT	Uric acid
Responses	↑P+B* ↑B* ↑B+M** ↑ P+M* ↑ M* ↑P+B+M*	↑P+B* ↑B+M** ↑P+M* ↑M**	↑B+M*	↑B*	↑M*	↑P+B* ↑M*

chol.=cholesterol; Mg=Magnesium; P=Phosphorus
↑=increase, N=6 in each group, *p<0.05, **p<0.01.

Tab. 2. Responses of oxidative stress parameters (FRAP, TBARS, GR) of Japanese quails exposed to single and combined doses of paraoxon (P), bromadiolone (B), and microcystins in cyanobacterial biomass (M) when compared against the healthy control group (C).

	Brain	Heart	Lung	Liver	Kidney	Muscle
FRAP	↑M* ↑P+B+M**	↑P+B+M**	↑M** ↑P+B+M**			↑P+B+M*
TBARS	↑P+B+M**	↑B* ↑B+M* ↑M** ↓P+B+M**	↑B+M* ↑P+M*	↑P+M* ↑M*	↑B+M* ↑P+M** ↑M*	↑M** ↓P+B+M**
GR	↓P+B+M*		↓P* ↓P+B** ↓B** ↓B+M* ↓P+M** ↓M** ↓P+B+M**	↓B*	↓P* ↓B** ↓B+M** ↓P+M** ↓M** ↓P+B+M**	

↑=increase, ↓=decrease, N=6 in each group, *p<0.05, **p<0.01.

was measured in the central nervous tissue of birds from the P+B+M group only. Activity of glutathione reductase was found to decrease in brain, lung, liver and kidney (cf. Table 2). The triple exposure group was the only affected one by the decrease in GR activity in the brain tissue.

As shown in the component score plot (Figure 1A), the principal component analysis of antioxidant/oxidative damage parameters in tissues clearly separated the triple exposure group (P+B+M) from all single and double exposure groups and the control. The component weights of parameters used for the principal component analysis document that the separation was driven by the modification of FRAP levels in heart and brain and the cardiac TBARS level, in particular (Figure 1B).

DISCUSSION

The research presented in this toxicological study indicates that adverse effects of cyanobacterial biomass containing microcystins, paraoxon as a cholinesterase-inhibitor and bromadiolone as an anticoagulant can be more severe in birds following combined exposure. Modulations of biochemical parameters were observed in Japanese quails used as an avian model species exposed to sub-lethal doses. While both single and double combinations of toxicants induced responses in individual biochemical parameters measured and evaluated using univariate analysis, those in the triple exposure were most extensive. Importantly, the multivariate principal component analysis confirmed marked joint effects in the overall pattern of antioxidant/oxidative stress responses of the triple exposure group.

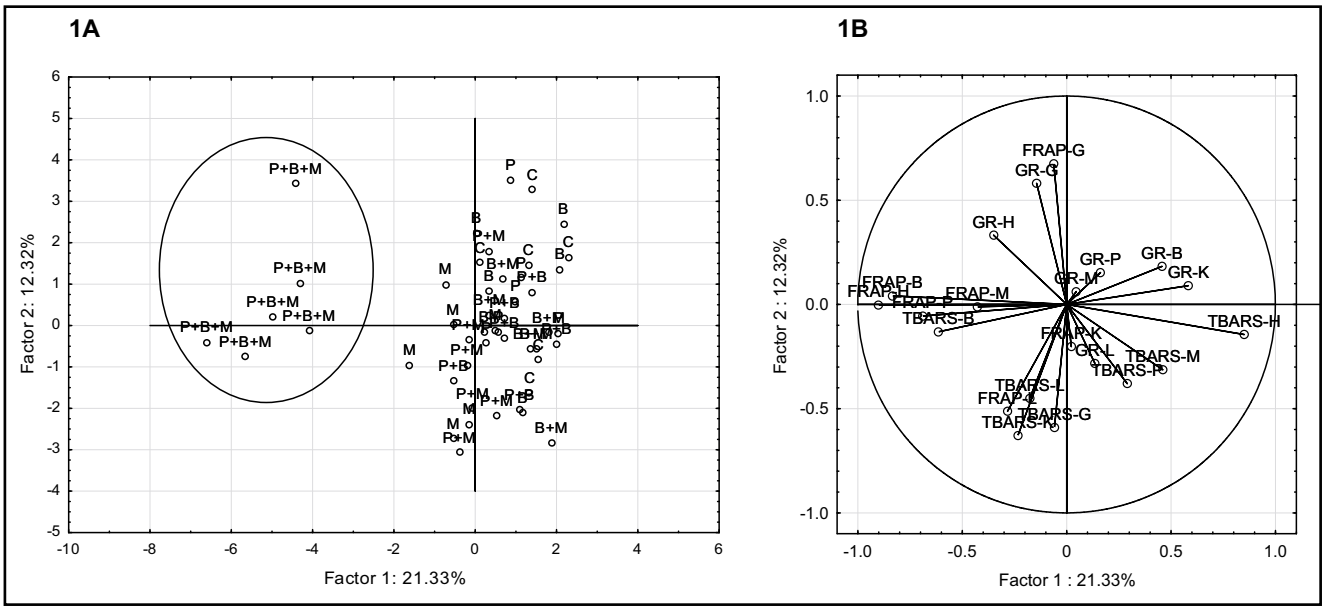

Fig. 1. Component score (1A) and component weight (1B) plots from principal component analysis. Group labels: control (C), paraoxon (P), bromadiolone (B), and microcystins in cyanobacterial biomass (M). Abbreviations: L=liver, K=kidney, H=heart, B=brain, P=pulmonary tissue, G=gonads, M=muscle, GR: glutathione reductase, TBARS: lipid peroxidation, FRAP: ferric reducing antioxidant power.

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This finding corresponds with the supposition based on our previous studies reporting enhancement of avian toxicity after a combination of single sub-lethal effects of microcystins, lead and Newcastle vaccination (Paskova *et al.* 2011; Pikula *et al.* 2010) or increased mortality in the grey partridge flock infected by *Mycoplasma gallisepticum* due to co-infection by another infectious agent (Vitula *et al.* 2011). Likewise, European brown hares exposed to the acetylcholinesterase inhibitor paraoxon were more susceptible to infection by a virulent *Francisella tularensis* subsp. *holarctica* strain and showed a rapid onset of clinical signs and death (Bandouchova *et al.* 2011).

The data also show that antioxidant/oxidative stress parameters were more sensitive, yet unspecific, indicators of avian exposure to toxins when compared with standard plasma biochemistry. Similar findings concerning the efficiency of activation of the antioxidant system in birds were reported earlier (Paskova *et al.* 2008, 2011). Interestingly, it seemed that, due to their physiological preparation for the oxidative stress associated with hatching, Japanese quail chicks were even better able to cope with the cyanobacterial-biomass-induced oxidative stress than adults (Peckova *et al.* 2009). Oxidative stress is involved in the adverse action of many stressors. For example, oxidative stress and immune responses of birds to infectious agents are closely related (Costantini & Moller 2009; Vitula *et al.* 2011).

Modulations of the antioxidant system were measured using two parameters: the ferric reducing antioxidant power, and glutathione reductase. The ferric reducing antioxidant power is a clinical marker of the total antioxidant capacity that depends on non-enzymatic antioxidants such as ascorbic and uric acids, bilirubin, vitamin E, α-tocopherol, and albumin (Benzie & Strain 1996; Pohanka *et al.* 2009). While lipid peroxidation is a measure of oxidative damage to membrane lipids, glutathione reductase, an enzyme that reduces the disulfide form to the sulfhydryl glutathione, is one of glutathione related indicators of oxidative stress (Halliwell & Gutterdige 1999; Paskova *et al.* 2008; Paskova *et al.* 2011). Another glutathione related enzyme, glutathione-*S*-transferase, is responsible for detoxification of microcystins as it catalyses their conjugation with glutathione (Pflugmacher *et al.* 1998).

While primary effects of the toxicants used were only sub-lethal, additional adverse actions were evaluated in this combination toxicology study. Avian oral exposure to microcystins results in hepatotoxicity (Alonso-Andicoberry *et al.* 2002; Lopez-Rodaz *et al.* 2008). The protective response, however, is associated with induction of the antioxidative system components (Paskova *et al.* 2008). The primary mode of action of paraoxon is irreversible inhibition of acetylcholinesterase and impairment of the breakdown of the neurotransmitter acetylcholine (Pohanka *et al.* 2010; Pohanka *et al.* 2011). The resulting accumulation of acetylcholine in synapses overstimulates muscarinic and nicotinic receptors. Apart from this, however, organophosphates can elicit generation of oxidative stress by disturbing glutathione homeostasis (Ojha & Srivastava 2012). Anticoagulants affect mainly vitamin K-dependent processes in liver, but changes in the activity of antioxidant enzymes such as superoxide dismutase and catalase were also reported (Belij *et al.* 2012).

Eutrophication of aquatic ecosystems and extensive use of pesticides in agriculture makes exposure of animals unavoidable and results in a growing number of poisoning events. Experiments employing combined exposures to multiple stressors can provide environmentally relevant data (Pikula *et al.* 2010). However, contrary to the traditional toxicological studies that focus on single stressor toxicity and standard endpoints such as the median lethal dose, sub-lethal responses of the avian protective system were evaluated in the present study. The joint effects in the overall pattern of antioxidant/oxidative stress responses of the triple exposure group were probably due to the fact that all the toxins target to some degree the liver, i.e., the general detoxifying organ with a major metabolic role in birds. As the present experiment was designed to examine acute or sub-acute effects, further studies are necessary to examine the longer-term health deterioration due to the sub-lethal exposure in birds.

CONCLUSION

While the study contributes to the understanding of the pathogenic mechanisms of combined sub-lethal exposure to natural toxins and agrochemicals in birds, the experimental data may be used for risk assessment of environmental pollution and evaluation of findings of low concentrations of contaminants in wild birds.

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Potential Conflicts of Interest: *None disclosed.*

REFERENCES

1 Alonso-Andicoberry C, Garcia-Villada L, Lopez-Rodas V, Costas E (2002). Catastrophic mortality of flamingos in a Spanish national park caused by cyanobacteria. Vet Rec. **151**: 706–707.
2 Babica P, Kohoutek J, Blaha L, Adamovsky O, Marsalek B (2006). Evaluation of extraction approaches linked to ELISA and HPLC for analyses of microcystin-LR, -RR and -YR in freshwater sediments with different organic material contents. Anal Bioanal Chem. **285**: 1545–1551.
3 Bandouchova H, Pohanka M, Kral J, Ondracek K, Osickova J, Damkova V, Vitula F, Treml F, Pikula J (2011). Effects of sublethal exposure of European brown hares to paraoxon on the course of tularemia. Neuroendocrinol Lett. **32**: 77–83.

4 Beklova M, Krizkova S, Supalkova V, Mikelova R, Adam V, Pikula J, Kizek R (2007). Determination of bromadiolone in pheasants and foxes by differential pulse voltammetry. I J Environ Anal Chem. **87**: 459–469.
5 Belij S, Miljkovic D, Popov A, Subota V, Timotijevic G, Slavic M, Mirkov I, Kataranovski D, Kataranovski M (2012). Effects of subacute oral warfarin administration on peripheral blood granulocytes in rats. Food Chem Toxicol. **50**: 1499–1507.
6 Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': The FRAP assay. Anal Biochem. **239**: 70–76.
7 Berny P (2007). Pesticides and the intoxication of wild animals. J Vet Pharmacol Ther. **30**: 93–100.
8 Carson R (1962). Silent spring. Boston: Houghton Mifflin.
9 Costantini D, Moller AP (2009). Does immune response cause oxidative stress in birds? A meta-analysis. Comp Biochem Physiol A Mol Integr Physiol. **153**: 339–344.
10 Damkova V, Sedlackova J, Bandouchova H, Peckova L, Vitula F, Hilscherova K, Paskova V, Kohoutek J, Pohanka M, Pikula J (2009). Effects of cyanobacterial biomass on avian reproduction: a Japanese quail model. Neuroendocrinol Lett. **30** (Suppl 1): 205–210.
11 Damkova V, Paskova V, Sedlackova J, Bandouchova H, Hilscherova K, Novotny L, Peckova L, Vitula F, Pohanka M, Pikula J (2011). Testicular toxicity of cyanobacterial biomass in Japanese quails. Harmful Algae. **10**: 612–618.
12 Fry DM (1995). Reproductive effects in birds exposed to pesticides and industrial chemicals. Environ Health Perspect. **103**: 165–71.
13 Guitart R, Sachana M, Caloni F, Croubels S, Vandenbroucke V, Berny P (2010). Animal poisoning in Europe. Part 3: Wildlife. Vet J. **183**: 260–265.
14 Halliwell B, Gutterdige JMC (1999). Free radicals in biology and medicine. Oxford: Oxford University Press.
15 Krienitz L, Ballot A, Kotut K, Wiegand C, Pütz S, Metcalf J S, Codd G A, Pflugmacher S (2002). Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. FEMS Microbiol Ecol. **43**: 141–148.
16 Krizkova S, Beklova M, Pikula J, Adam V, Horna A, Kizek R (2007). Hazards of Secondary Bromadiolone Intoxications Evaluated using High-performance Liquid Chromatography with Electrochemical Detection. Sensors. **7**: 1271–1286.
17 Lopez-Rodas V, Maneiro E, Lanzarot MP, Perdigones N, Costas E (2008). Mass wildlife mortality due to cyanobacteria in the Doñana National Park, Spain. Vet Rec. **162**: 317–318.
18 Ojha A, Srivastava N (2012). Redox imbalance in rat tissues exposed with organophosphate pesticides and therapeutic potential of antioxidant vitamins. Ecotox Environ Safe. **75**: 230–241.
19 Paskova V, Adamovsky O, Pikula J, Skocovska B, Bandouchova H, Horakova J, Babica P, Marsalek B, Hilcherova K (2008). Detoxification and oxidative stress responses along with microcystins accumulation in Japanese quail exposed to cyanobacterial biomass. Sci Total Environ. **398**: 34–47.
20 Paskova V, Paskerova H, Pikula J, Bandouchova H, Sedlackova J, Hilscherova K (2011). Combined exposure of Japanese quails to cyanotoxins, Newcastle virus and lead: Oxidative stress responses. Ecotox Environ Safe. **74**: 2082–2090.
21 Peckova L, Bandouchova H, Hilscherova K, Damkova V, Sedlackova J, Vitula F, Paskova V, Pohanka M, Kohoutek J, Pikula J (2009). Biochemical responses of juvenile and adult Japanese quails to cyanobacterial biomass. Neuroendocrinol Lett. **30**: 199–204.
22 Pflugmacher S, Wiegand C, Oberemm A, Beattie KA, Krause E, Codd GA, Steinberg CE (1998). Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR: the first step of detoxication. Biochim Biophys Acta.**1425**: 527–533.
23 Pikula J, Bandouchova H, Hilscherova K, Paskova V, Sedlackova J, Adamovsky O, Knotkova Z, Lany P, Machat J, Marsalek B, Novotny L, Pohanka M, Vitula F (2010). Combined exposure to cyanobacterial biomass, lead and the Newcastle virus enhances avian toxicity. Sci Total Environ. **408**: 4984–4992.
24 Pohanka M, Bandouchova H, Sobotka J, Sedlackova J, Soukupova I, Pikula J (2009). Ferric reducing antioxidant power and square wave voltammetry for assay of low molecular weight antioxidants in blood plasma: Performance and comparison of methods. Sensors. **9**: 9094–9103.
25 Pohanka M, Karasova JZ, Kuca K, Pikula J, Holas O, Korabecny J, Cabal J (2010). Colorimetric dipstick for assay of organophosphate pesticides and nerve agents represented by paraoxon, sarin and VX. Talanta. **81**: 621–624.
26 Pohanka M, Snopkova S, Havlickova K, Bostik P, Sinkorova Z, Fusek J, Kuca K, Pikula J (2011). Macrophage-assisted inflammation and pharmacological regulation of the cholinergic anti-inflammatory pathway. Curr Med Chem. **18**: 539–551.
27 Rattner BA (2009). History of wildlife toxicology. Ecotoxicology.**18**: 773–783.
28 Sanchez-Barbudo IS, Camarero PR, Mateo R (2012). Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. Sci Total Environ. **420**: 280–288.
29 Skocovska B, Hilscherova K, Babica P, Adamovsky O, Bandouchova H, Horakova J, Knotkova Z, Marsalek B, Paskova V, Pikula J (2007). Effects of cyanobacterial biomass on the Japanese quail. Toxicon. **49**: 793–803.
30 Strum KM, Hooper MJ, Johnson KA, Lanctot RB, Zaccagnini ME, Sandercock BK (2010). Exposure of nonbreeding migratory shorebirds to cholinesterase-inhibiting contaminants in the western hemisphere. Condor. **112**: 15–28.
31 Vitula F, Peckova L, Bandouchova H, Pohanka M, Novotny L, Jira D, Kral J, Ondracek K, Osickova J, Zendulkova D, Rosenbergova K, Treml F, Pikula J (2011). *Mycoplasma gallisepticum* infection in the grey partridge (*Perdix perdix*): outbreak description, histopathology, biochemistry and antioxidant parameters. BMC Vet Res. **7**: 34.

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