Chemosphere 168 (2017) 163–170

Contents lists available at ScienceDirect

Chemosphere

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

Mixtures of herbicides and metals affect the redox system of honey bees

Catherine Jumarie*, Philippe Aras, Monique Boily

Département des Sciences Biologiques, Centre TOXEN, Université du Québec à Montréal, Montréal, Québec, Canada

HIGHLIGHTS

- Co-exposure to atrazine, glyphosate and Cd lowered bee carotenoid contents.
- Co-exposure to high levels of Fe decreased the accumulation of Cd in bees.
- Orally ingested Fe was bioconcentrated in bees.
- Fe induced high level of lipid peroxidation.
- Bees exposed to Fe had lower levels of 9-*cis*-RA and 13-*cis*-RA.

ARTICLE INFO

Article history:
Received 7 September 2016
Received in revised form
13 October 2016
Accepted 14 October 2016
Available online 22 October 2016

Handling Editor: David Volz

Keywords:
Apis mellifera
Metals
Atrazine
Glyphosate
Retinoids
Carotenoids
Lipid peroxidation

ABSTRACT

The increasing loss of bee colonies in many countries has prompted a surge of studies on the factors affecting bee health. In North America, main crops such as maize and soybean are cultivated with extensive use of pesticides that may affect non-target organisms such as bees. Also, biosolids, used as a soil amendment, represent additional sources of metals in agroecosystems; however, there is no information about how these metals could affect the bees. In previous studies we investigated the effects of environmentally relevant doses of herbicides and metals, each individually, on caged honey bees. The present study aimed at investigating the effects of mixtures of herbicides (glyphosate and atrazine) and metals (cadmium and iron), as these mixtures represent more realistic exposure conditions. Levels of metal, vitamin E, carotenoids, retinaldehyde, *at*-retinol, retinoic acid isomers (9-*cis* RA, 13-*cis* RA, *at*-RA) and the metabolites 13-*cis*-4-oxo-RA and *at*-4-oxo-RA were measured in bees fed for 10 days with contaminated syrup. Mixtures of herbicides and cadmium that did not affect bee viability, lowered bee α- and β-carotenoid contents and increased 9-*cis*-RA as well as 13-*cis*-4-oxo-RA without modifying the levels of *at*-retinol. Bee treatment with either glyphosate, a combination of atrazine and cadmium, or mixtures of herbicides promoted lipid peroxidation. Iron was bioconcentrated in bees and led to high levels of lipid peroxidation. Metals also decreased zeaxanthin bee contents. These results show that mixtures of atrazine, glyphosate, cadmium and iron may affect different reactions occurring in the metabolic pathway of vitamin A in the honey bee.

1. Introduction

In many countries, the overwintering losses of honey bee colonies have risen beyond the 20% level during the last decades (Aizen and Herder, 2009). This concern prompted numerous studies to identify the causes affecting the health of bees, among them agricultural practices that may modify the quality of the bee environment and its nutritional status and hence its development, immune system and neuronal system (Wu et al., 2011; Boily et al., 2013; Pettis et al., 2013). In the province of Quebec, wide-row corn and soybean are the main crops, together occupying 665 kha in 2012 and consuming 46% of the pesticide use in 2008 (ISQ and MAPAQ, 2013; Gorse and Rivard, 2011). Glyphosate and atrazine are still widely used in these crops in the United States and Canada. They are available for the foraging bees from pollen, nectar, water, and dust (Krupke et al., 2012). Also, in addition to natural and chemical fertilizers, biosolids (municipal wastewater treatment

* Corresponding author. Département des sciences biologiques, Centre TOXEN, Université du Québec à Montréal, C.P. 8888, succ Centre-ville, Montréal, QC H3C 3P8, Canada.
E-mail address: jumarie.catherine@uqam.ca (C. Jumarie).

http://dx.doi.org/10.1016/j.chemosphere.2016.10.056
0045-6535/

sludge) are used as soil amendment to stimulate rooting. Although this practice follows restriction criteria based on the sludge's metal content, these biosolids represent a significant source of metals as they can contain up to 3 mg/kg cadmium (Cd) and 150 mg/kg lead (MDDEP, 2012). Metals in soil are available to plants, and therefore to foraging bees. For example, Cd accumulates in all parts of the maize (*Zea mays*) plant (Xu et al., 2013; Wang et al., 2016) and may exhibit a five-fold concentration in the pollen of the partridge pea (*Anisacanthus linearis*) compared to the flower part of the plant (Henson et al., 2013). Very few is known about how these metal contents may affect pollinators. Nonetheless, pesticides and metals found in pollen are brought back to the hive (Mullin et al., 2010; Lambert et al., 2012), and metals were shown to affect the overall hive health status, as revealed by lower honey production, increased dead pupae in the capped cells, and lowered relative pupae growth index (Di et al., 2016; Hladun et al., 2016). Atrazine, glyphosate and metals may induce oxidative stress, in other words, an imbalance between the level of pro-oxidant chemical species and the antioxidative defense, which may result in lipid peroxidation (Thornton et al., 2010; Jasper et al., 2012; Keshk et al., 2014; Nwani et al., 2010).

Alpha-tocopherol (vitamin E) and carotenoids, provided by diet, protect against oxidative damage. In vertebrates, carotenoids can also be oxidized into retinaldehyde (RALD), which in turn may be reversibly reduced to form all-*trans* retinol (ROH) or be oxidized into retinoic acid (RA) by a unidirectional pathway. In insects as well as in vertebrates, retinoids are essential for a number of biological functions including vision, reproduction and immune system response. In *Drosophilia*, imbalanced RA levels may lead to blindness or developmental malformations (von Lintig et al., 2001; Nakamura et al., 2007). For bees, pollen represents an important source of α- and β-carotene (Mărgăoan et al., 2014), which are converted into retinoids by the carotenoid isomeroxygenase NinaB, responsible for both the oxidative cleavage and the isomerization of carotenoids (Oberhauser et al., 2008). In vertebrates, there are three isomers for RA: all-*trans* retinoic acid (*at*-RA), 9-*cis* retinoic (9-*cis*-RA) and 13-*cis* retinoic acid (13-*cis*-RA). The transcriptional activity of RA isomers involves *at*-RA, 9-*cis*-RA and 13-*cis*-RA binding to the nuclear receptor RAR, whereas the RXR receptor reacts only with 9-*cis*-RA (Bastien and Rochette-Egly, 2004). The affinity of the 13-*cis*-RA isomer for the RAR receptor is much lower compared to that of *at*-RA; however, the possibility of an intracellular isomerization of 13-*cis*-RA to *at*-RA has been suggested (Tsukada et al., 2000; Veal et al., 2002). The cellular level of 13-*cis*-RA may, indirectly, play a role in RA activity. In vertebrates, RA isomers are converted into various polar metabolites, including *at*-4-oxo-RA and 13-*cis*-4-oxo-RA. The CYP26A1/B1/C1 isoforms of the CYP450 superfamily play a major role in the first step of the oxidative catabolism of *at*-RA leading to the formation of *at*-4-OH-RA (Armstrong et al., 2005; Petkovitch, 2001). Thus, controlling synthesis, isomerization and degradation finely regulates RA signaling.

In a previous study we have shown that both β-carotene and retinol levels decreased when bees were exposed to atrazine or glyphosate via contaminated syrup (Hedrei Helmer et al., 2015). We have also shown that Cd, at environmentally relevant concentrations in the syrup, is available for bees and increased the level of metallothionein-like proteins (Gauthier et al., 2016). The aim of the present study was to pursue our investigation of the effects of herbicides and metals on bee health by testing more realistic exposure conditions, being herbicide and metal mixtures. Carotenoids were measured for their antioxidant properties as well as for their involvement in RA metabolism. Retinoic acid was studied because of its crucial role in development and neuronal function. Cadmium and iron (Fe) were chosen for the following reasons: i) both metals may promote oxidative stress (Wu et al., 2016); ii) one of our previous studies shows the induction of stress proteins in bees exposed to Cd; iii) Cd may interfere with RA metabolism in some species (Cui and Freedman, 2009; Lee et al., 2006); iv) up to 850 ppm Fe were measured in samples of pollen from maize fields.

2. Material and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT), malondialdehyde (MDA), carotenoids (lutein zeaxanthin, α- and β-cryptoxanthin, α- and β-carotene), retinol (ROH), vitamins A (all-*trans*-retinol) and E (α-tocopherol) standards were purchased from Sigma-Aldrich Ltd. (Oakville, ON, Canada). Antipain dihydrochloride, pepstatin A, tris-(2-carboxyethyl)phosphine hydrochloride (TCEP), 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonic acid ammonium (SBD-F) and 2-thiobarbituric acid (TBA) were purchased from Sigma-Aldrich Ltd. HPLC-grade solvents were used. Cadmium, as $CdCl_2$, was purchased from Sigma-Aldrich Ltd., whereas Fe, as $FeCl_2 \cdot 4H_2O$, was bought from Alfa Aesar – Thermo Fisher Scientific (Ward Hill, MA, US). Commercial formulations of atrazine (Aatrex®: 480 g active matter/L) and glyphosate (Credit Extreme®: 540 g active matter/L) were purchased from Les Moulins Mondou (Mirabel, QC, Canada).

2.2. Honey bee exposure

Honey bees (from the same healthy beehive) were taken from the frame without brood during the summer of 2014 and exposed to both herbicides and Cd (August 12–22) as well as to combinations of Cd and Fe (August 25 to September 4). Bees were placed in acrylic cages (3 cages, 32–38 bees/cage) and were fed a sucrose solution (50% w/w) ad libitum, as already performed in a prior study (Hedrei Helmer et al., 2015). Bees were exposed to syrup containing 0.03 mg/L Cd, 0.12 mg/L atrazine or glyphosate, alone or as mixtures. The exposure level to metals was based on the metal concentrations measured in maize pollen sampled in 2012, as determined in a previous study (Gauthier et al., 2016): Cd and Fe contents ranged from 0.01 to 0.03 ppm and from 90 to 850 ppm, respectively. The level of atrazine and glyphosate was based on herbicide concentrations measured in crops and vegetation (Krupke et al., 2012) and was estimated considering the quantity of active matter in the respective commercial formulations. Considering the daily consumption of syrup of 41 µl/bee estimated previously (Hedrei Helmer et al., 2015), the level of herbicides in the syrup led to a daily dose of 5.0 ng/bee, which represents less than 1% of the LD_{50} for atrazine and glyphosate (ARLA, 2007). Control cages were supplied with a sucrose solution only. The control and contaminated sugar solutions were prepared about 10 days prior to exposure, stored at −20 °C and thawed at room temperature before use. The sugar solutions of all cages (three replicates per experimental condition) were changed daily for the duration of the 10-day test. Cages were equipped with two feeders for syrup (1.5-ml capacity each). These were weighed before and after daily changes to estimate the consumption of syrup per cage/day adjusted for dead bees. Mortality was recorded every 24 h. After 10 days, the surviving bees were anesthetized/euthanized by placing the cages in an insulated container with dry ice for 5 min and stored at −80 °C until analysis. Pools of bees ($n = 7$ to 10; randomly mixed between replicates for each dose of contaminant) were analyzed for retinoids, carotenoids, α-tocopherol, peroxidation of lipids and protein content determination.

2.3. Metal content analyses

Metal concentrations in contaminated syrup as well as metal

levels in bees were verified by chemical analysis by the Centre d'expertise en analyse environnementale du Québec (CEAEQ, QC, Canada). In all cases, the metal content in the syrup fell within the 90% confidence interval of the nominal concentration. The pools of bees (three pools of 6 bees per dose) were lyophilized and tissues were digested in a microwave oven (195 °C, 15 min) with both HNO_3 and HCl acids (3:1) along with blanks and certified reference samples (lobster hepatopancreas reference material for trace metal, TORT-2 and Fish Protein Certified Reference Material for Trace Metals, DORM-3, National Research Council of Canada). Samples were allowed to cool for 30 min and metals were measured by ICP-MS. Metal bioconcentration ratios (bee metal level/syrup metal concentration) were estimated as followed. Considering that humidity accounts for 70% of the bee mass, and a mean mass of 0.1 g/bee, metal content expressed as μg/g dry weight (A) was expressed as μg/g wet weight (B = A × 0,3) and then converted to metal content per bee (C = B × 0.1).

2.4. Retinoids, carotenoids and α-tocopherol content determination

Seven to 10 pools of two or three whole bees were weighed, rinsed with 100% acetone and homogenized on ice in a glass tube (12-ml screw top borosilicate) with four volumes of daily fresh homogenization solution pH 7.5 (PBS containing 0.05 mM antipain, 5 μM pepstatin A and 0.5% ascorbic acid). Homogenates (1.5 ml) were mixed with 1 ml of MeOH (0.1% BHT), vortexed for 30 s, and extracted three times with hexane (0.1% BHT):acetone (50:50). After each addition of the hexane:acetone solution, the tube was vortexed for 90 s and centrifuged for 5 min at 1625 × *g*. The supernatant (1.7 ml) was collected and distributed evenly between two 5-ml disposable glass tubes and evaporated to dryness for 10 min in a vacufuge (EppendorfTM, Fisher Scientific, Ottawa, Canada) at 45 °C. This procedure was repeated with the second extraction (1.5 ml) and the third extraction (1.7 ml). A volume of 2 ml bee homogenate extract was centrifuged at 4 °C, 9600 × g for 10 min and the supernatant was aliquoted and frozen at −80 °C until used for subsequent measurements of lipid peroxidation. For carotenoids, retinoids and α-tocopherol content determination, 2 ml of the homogenate was transferred into two 5-ml glass tubes and centrifuged to dryness at 4 °C, 9600 × *g* for 10 min. The residue of each tube was resuspended in 100 μl acetonitrile and vortexed for 30 s. From each tube, 80 μl were collected and pooled in a 5-ml disposable glass tube, vortexed for 10 s and injected in the HPLC systems. Two injections were performed simultaneously in two reversed-phase HPLC systems (Water Corporation, Milford, MA) connected to Empower Pro software (version 5.0), using a model 510 pump and a model 7725i Rheodyne injector.

Carotenoids, namely lutein, zeaxanthin, α- and β-cryptoxanthin and α- and β-carotene, were detected at 445 nm and α-tocopherol at 292 nm using the chomatographic methods described previously (Hedrei Helmer et al., 2015) with 30-μl sample extracts. Retinoids were detected at 325 nm using a procedure adapted from Hedrei Helmer et al. (2015) allowing the detection of retinaldehyde and the discrimination between RA isomers. The retinoids were separated on an Ace C18 analytical column, 4.6 × 150 mm, 3 μm (Canadian Life Science, Peterborough, ON, Canada), using gradient elution with the initial solvent A: ACN-H_2O-formic acid (60:40:0.1) changing linearly over a 5-min period to the final solvent B: ACN-H_2O-formic acid (85:15:0.1). After 15 min on solvent B, a 3-min linear gradient was used to return to the 100% solvent A. The flow rate was 1.0 ml/min for the total 26-min elution time. Under these conditions, the retention times for 13-*cis*-4-oxo-RA, *at*-4-oxo-RA, 13-*cis*-RA, 9-*cis*-RA, *at*-RA and *at*-ROH were 5.6, 6.2, 11.9, 12.5, 13.9 and 14.2 min, respectively. Retinaldehyde isomers (*trans* and *cis*) co-eluted (retention time: 15.2 and 15.6). The two peaks (areas under the curve) were then summed and referred to in the text as retinaldehyde (RALD). Peaks of HPLC chromatograms for RALD, ROH as well as *at*-RA, 9-*cis*-RA and 13-*cis*-RA were identified with commercial standards. The metabolites *at*-4-oxo-RA and 13-*cis*-4-oxo-RA were obtained by irradiation of *at*-RA in a heptane-iodine solution as performed previously (McKenzie et al., 1979; Solari et al., 2010). Honey bee tissue extraction and chromatography were performed under yellow incandescent light to prevent isomerization of the compounds.

2.5. Analysis of lipid peroxidation

The measurement of oxidative stress was evaluated as described in Hedrei Helmer et al. (2015) by measuring the levels of thiobarbituric acid reactive substances (TBARS) as by-products of lipid peroxidation, notably malondialdehyde, in 200-μl bees' homogenates.

2.6. Protein content determination

The protein content in each sample (supernatants obtained following the first centrifugation of homogenates) was determined by the Bradford method (Bradford, 1976).

2.7. Statistical analysis

The survival of bees during the 10-days exposure was compared between control and treatment groups using pairwise Kaplan-Meier analysis where dead bees were recorded and censored. Consumption of syrup was tested using general linear model (GLM)-based two-way repeated measures (time and treatment design including a “time x treatment” interaction term). Bees' mass and metal contents values were compared using a GLM one-way analysis of variance followed by an adjusted pairwise Bonferroni test. Values for the bioconcentration ratio were compared using the Kruskal-Wallis non-parametric test followed by Dunn's test. Mean concentrations of retinoids, carotenoids, α-tocopherol, TBARS and protein as well as bee mass were compared by a GLM one-way analysis of variance, followed by the Dunnett *t* post hoc test against the control group. When transformation failed to normalize the distribution of the data, non-parametric tests were used. All statistical tests were performed with SPSS® Statistic 18.0 software (IBM® Corporation, Armonk, NY, USA).

3. Results and discussion

The exposure conditions follow the OECD (Organisation for Economic Co-operation and Development) recommendation that protocols must ensure a minimum of 90% viability in the control groups for bees (OECD, 1998). Although this recommendation only concerns acute toxicity, the mean 10-day survival of control bees in our study was 96.6 ± 2.1%. No significant differences were observed between survival of control bees and bees treated with atrazine, glyphosate and Cd (χ^2 13.59; p = 0.059, Supplementary data). However, survival was significantly different for bees exposed to metals mixture (χ^2 29.15; $p < 0.001$). The survival was lower in bees fed 200 ppm Fe, alone or in combination with 0.03 ppm Cd, compared to control bees (Fig. 1). Data obtained with herbicides are in accordance with our previous studies showing that a daily dose of 5 ng/bee of atrazine or glyphosate or Cd-contaminated syrup (0.03 ppm) did not modify bees' survival (Hedrei Helmer et al., 2015; Gauthier et al., 2016). The present study reveals that mixtures of herbicides and Cd do not have an additional effect on bees' mortality. Similar results were obtained with Fe and Cd: whatever the level of Fe in the contaminated syrup, Cd did not increase Fe-

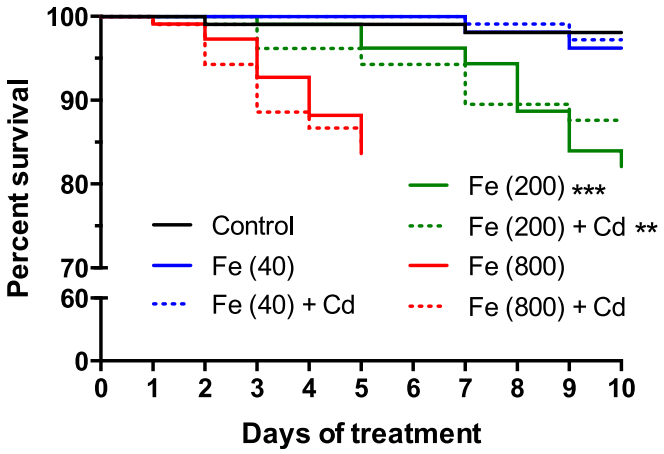

Fig. 1. Survivorship for honey bees exposed for 10 days to 40 or 200 ppm Fe alone or in combination with Cd (0.03 ppm) or exposed for 5 days to 800 ppm Fe with or without Cd (0.03 ppm). Number of bees for each groups: Ctrl: 105, Fe(40):106, Fe(200):106, Fe(800):110, Fe(40) + Cd: 108, Fe(80) + Cd: 105, and Fe(800) + Cd: 105. Survival curves were compared against the control group using pairwise Kaplan-Meier analysis (log-rank tests): Statistically different compared to control: $^{**}p < 0.01$; $^{***}p < 0.001$.

induced bee mortality. Exposure to 800 ppm Fe was stopped on day 5 because of a high level of mortality, probably related to too low consumption of syrup. Indeed, in the series of experiments with metal mixtures, the mean daily consumption for the control syrup over the 10-day exposure was 33.6 ± 6.0 µl/bee, whereas consumption of syrup containing 800 ppm Fe (±Cd) was: 20.6 ± 0.4, 10.9 ± 0.4, 9.3 ± 0.4, 10.4 ± 0.4 and 4.4 ± 0.1 µl/bee on day 1, 2, 3, 4 and 5, respectively. Hence, and contrary to what was observed for Cd, Al and Pb (Gauthier et al., 2016), Fe-contaminated syrup seems to be "repellent" to bees. Excluding the data obtained with 800 ppm Fe, syrup consumption increased slightly during the 10-day exposure, but no significant differences were noted between treatments and time including the interaction term "treatment x time" ($D_{36,90} = 1.25$; $p = 0.201$). Similar results were obtained with the series of experiments conducted with atrazine, glyphosate and Cd ($D_{63,144} = 1.14$; $p = 0.263$) (Supplementary data).

Bee metal content values suggest that the 10-day accumulation of Fe does not increase linearly with increasing syrup Fe content (Table 1). Accumulation estimated on day 10 of exposure is the result of the cumulative daily absorption and of the bees' capacity to eliminate metals. Because more efficient mechanisms of elimination at a higher level of Fe are very unlikely, the non-linear 10-day accumulation as a function of Fe concentration would rather be related to the saturation of processes responsible for Fe absorption. Similar Fe contents in bees were obtained whatever the presence or the absence of Cd in the syrup, suggesting that Cd does not affect Fe accumulation or elimination under the experimental conditions. Cadmium levels in bees fed with the syrup "0.03 ppm Cd + 40 ppm Fe" were similar to that reported previously in bees fed with 0.03 ppm Cd alone (Gauthier et al., 2016). Indeed, after correction for the basal level of metal in control bees, an accumulation of ≈0.33 µg/g (dw) was obtained for Cd from syrup consumption, which is very close to the value of ≈0.30 µg/g (dw) estimated in the present study. However, significantly lower levels and bioconcentartion ratios were measured for Cd in the presence of 200 ppm Fe compared to 40 ppm Fe, suggesting that high concentrations of Fe could interfere with the accumulation processes of Cd. The mammalian proton-dependent divalent cation transporter NRAMP2 (DMT1/DCT1) has a broad selectivity (Gunshin et al., 1997), and competition between Fe and Cd for NRAMP2-mediated transport in intestinal cells has been demonstrated (Elisma and Jumarie, 2001). Malvolio (Mvl), the *Drosophila melanogaster* homologue of Nramp1/2, is expressed in the larva and adult alimentary canal of drosophila (Rodrigues et al., 1995; Folwell et al., 2006). The genome of *Apis mellifera* has not been completely annotated yet, but the gene Nramp2/Slc11a2 is ubiquitously expressed (Cellier et al., 1995), thus competition between Cd and Fe for the oral absorption is expected to occur also in the honey bee under some conditions.

Bioconcentration ratios estimated for Fe were similar to that of 3.2 ± 0.2 and 2.5 ± 0.2 previously found for Cd and Pb, respectively (Gauthier et al., 2016). Hence, Fe, such as Cd and Pb, would be poorly eliminated compared to the rate of absorption. It must be remembered that, because of the too high level of mortality, the bioconcentration ratios estimated with 800 ppm Fe (±Cd) are for a 5-day exposure. In this case, in addition to the putative saturation of absorption processes, the much lower syrup consumption is clearly responsible for the much lower bioconcentration ratios. Decreases in syrup consumption also led to significant mass loss. Indeed, bees fed with syrup containing 800 ppm Fe had significant

Table 1
Metal contents and bioconcentration measured in honey bees exposed for $10^{(1)}$ or $5^{(2)}$ days to increasing levels of Fe in the absence or the presence of 0.03 ppm Cd. Data are means ± SD estimated on 3 pools of 6 bees.

Metal (mg/L sugar syrup)	Syrup consumption (µl/bee) [min - max]	Fe (µg/g dry tissue)	Cd (µg/g dry tissue)	Bioconcentration ratio for Fe	Bioconcentration ratio for Cd
Control	33.6 ± 6.0 [26.0–45.2]	173 ± 16^{a}	0.07 ± 0.01^{ab}	–	–
Fe (40) $^{(1)}$	33.6 ± 6.0 [44.3–31.4]	527 ± 171^{a}	0.06 ± 0.02^{ab}	2.65 ± 1.23^{a}	–
Fe (200) $^{(1)}$	27.4 ± 4.1 [18.6–33.3]	1129 ± 206^{b}	0.06 ± 0.02^{a}	1.44 ± 0.31^{ab}	–
Fe (800) $^{(2)}$	11.1 ± 5.2 [4.4–20.3]	1059 ± 32^{b}	0.07 ± 0.01^{ab}	0.33 ± 0.01^{b}	–
Fe (40) + Cd $^{(1)}$	39.1 ± 3.6 [30.1–48.0]	531 ± 36^{a}	0.38 ± 0.03^{d}	2.69 ± 0.26^{a}	3.16 ± 0.25^{a}
Fe (200) + Cd $^{(1)}$	37.9 ± 5.8 [18.9–37.2]	1365 ± 142^{b}	0.30 ± 0.02^{c}	1.79 ± 0.21^{ab}	2.36 ± 0.23^{b}
Fe (800) + Cd $^{(2)}$	11.1 ± 5.4 [4.3–20.8]	1159 ± 179^{b}	0.11 ± 0.01^{b}	0.37 ± 0.07^{b}	0.43 ± 0.10^{c}
Statistic model		$D_{6,14} = 31.71$ $p < 0.001$	$D_{6,14} = 182.06$ $p < 0.001$	$KW = 14.49$ $p < 0.05$	$KW = 6.33$ $p < 0.05$

D: GLM one-way analysis of variance followed by adjusted pairwise Bonferroni test ($p < 0.05$).
KW: Kruskal-Wallis non-parametric test followed by Dunn's test ($p < 0.05$).
Data not sharing the same letters are statistically different.

lower mass on day 5 compared to those fed with 40 ppm Fe (whatever the presence or the absence of Cd) for 10 days (Fig. 2B). However, some treatments may influence the bee mass without altering the syrup consumption. Bees treated with a combination of atrazine, glyphosate and Cd had significant lower mass compared to bees exposed to atrazine alone (Fig. 2A). In the two series of experiments (with mixtures of herbicides or with metal mixtures), the mean value for bee total protein was 207 ± 26 mg/g of tissue. In the experiments with mixtures of herbicides, there was no significant differences between groups ($F = 1.17$; $p = 0.33$) while in the experiments with metal mixtures a significant model was computed ($F = 2.65$; $p < 0.05$) but a true significant difference was found only between the groups Fe (40) and Fe (800) ($p < 0.05$) (data not shown).

We have previously shown that atrazine and glyphosate may modify ROH levels in bees fed the same dose of 5 ng/bee for 10 days (Hedrei Helmer et al., 2015). However, we did not measure any of the RA isomers nor RALD, and we did not investigate the impact of mixtures on bee carotenoid and retinoid contents. In addition to RA, RALD quantification is important because of its involvement in RA synthesis. Recently, we improved our HPLC method and modified the mobile phase to be able to quantify RALD and RA isomers in same samples as for ROH and α-tocopherol. The present data show that atrazine, glyphosate or Cd alone did not lower the levels of α- or β-carotene compared to the control bees, but mixtures of herbicides and Cd decreased them (Table 2). Alpha- and β-carotene both found in pollen (Mărgăoan et al., 2014) can be converted into RALD to produce ROH or RA. Thus lower levels of carotenoids may reflect higher rates of metabolic cascade to form ROH and RA. The levels of ROH were similar whatever the exposure conditions. In all samples *at*-RA and *at*-4-oxo-RA contents were very low (ranging from 1.2 to 4.0 ng/g of tissue) and were only detected in a few samples excluding the possibility of statistical analysis (data not shown). However, the levels of 9-*cis*-RA and 13-*cis*-4-oxo-RA were higher in bees exposed to mixtures of herbicides alone or in combination with Cd, respectively. This suggests that RA isomerization and catabolism would be favored over ROH formation under these conditions. Conversion of carotenoids into RALD would be stimulated in response to the increased degradation of RA isomers. Whatever the bee treatment, RALD contents did not vary, suggesting kinetic coupling between RALD formation and conversion into either ROH or RA. Some xanthophylls are also precursors for vitamin A synthesis. None of the exposure conditions affected the levels of lutein, α-crytoxanthin and β-cryptoxanthin (data not shown), whereas higher and lower zeaxanthin concentrations were estimated in bees fed with syrups "ATZ + GLY" and "GLY + Cd," respectively. All these results suggest that mixtures of atrazine, glyphosate and Cd may affect some metabolic steps downstream of the RALD formation. They also emphasize the complex effects that herbicides and Cd may have on vitamin A metabolism. Contrary to herbicides, metal mixtures did not affect the levels of carotenoids and there was no evidence for perturbation of the RA metabolism (Table 3). However, in all cases, exposure to metal lowered bee zeaxanthin contents and significant differences compared to control values were obtained with 40 and 200 ppm Fe as well as with 200 ppm Fe + Cd. This metal effect was also clearly observed in the experiments with herbicides in which lowered zeaxanthin contents were measured in the presence of Cd. In accordance with what has been previously observed by Hedrei Helmer et al. (2015), β-carotene was the most abundant carotenoid (Tables 2 and 3). In the present study, similar control levels of carotenoids were measured in the two series of experiments (herbicide and metal mixtures). Bees of the herbicide groups were captured in mid-August, whereas bees of the metal groups were captured at the end of August. During this two week-period, no significant variation in pollen and nectar source is expected contrary to what was observed previously, where bees were captured from mid-July to the beginning of September (Hedrei Helmer et al., 2015).

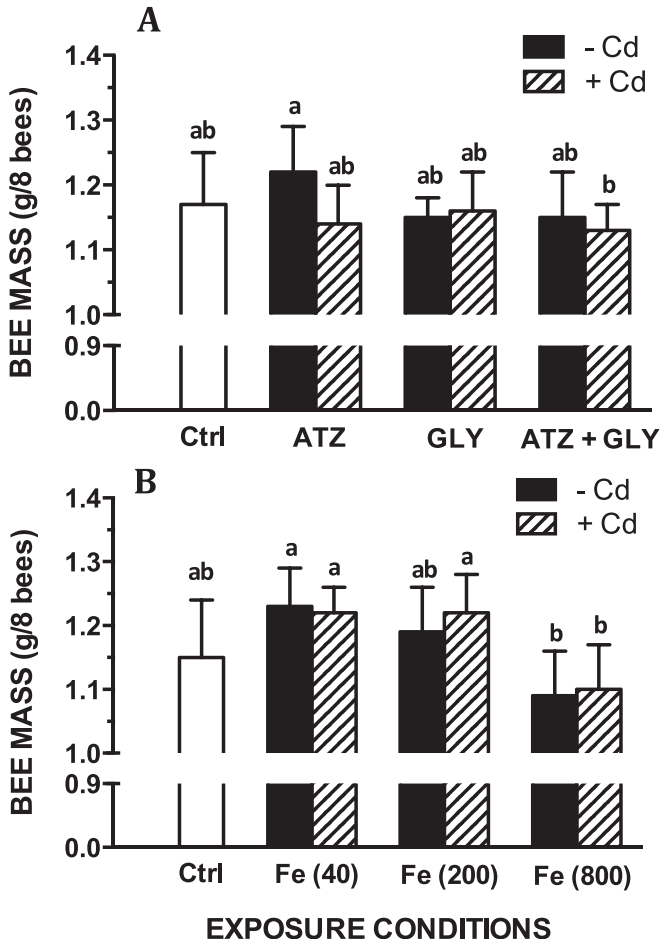

Fig. 2. Means ± SD of bee mass (g/8 bees) estimated from 9 to 10 pools of 8 bees exposed to: A) atrazine (ATZ, 5 ng/bee), glyphosate (GLY, 5 ng/bee) or a mixture of both in the absence (black columns) or the presence (dashed columns) of Cd (0.03 ppm); B) 40, 200 or 800 ppm Fe alone (black columns) or in combination with 0.03 ppm Cd (dashed columns). Exposure time was 10 days except for 800 ppm Fe (5 days). Data were compared using GLM one-way analysis of variance followed by adjusted pairwise Bonferroni test (A: $p < 0.05$; B: $p < 0.001$). Data with different letters are statistically different.

The health status of bees fed with metal or herbicide mixtures was also investigated by measuring lipid peroxidation using the TBARS assay. Bee treatment with glyphosate, a combination of atrazine and Cd or a mixture of herbicides led to significant higher levels of TBARS, from 30 to 40% relative to the control values (Fig. 3A). Atrazine and glyphosate have been shown to induce oxidative stress, including lipid peroxidation, in a number of vertebrates (Jasper et al., 2012; Keshk et al., 2014; Nwani et al., 2010). However, in a recent study conducted with *Drosophila melanogaster* fed with contaminated food, glyphosate up to 10 ppm failed to induce lipid peroxidation (de Aguiar et al., 2016). Considering the mean daily syrup consumption of 34.9 ± 9.5 µl/bee measured with the glyphosate-contaminated syrup in our study, the daily dose of 5 ng/bee corresponds to 0.143 ppm, which is two orders of magnitude lower compared to the study conducted by de Aguiar et al. (2016). However, in this study performed with *Drosophila melanogaster*, the maximal duration of the exposure was 96 h. Thus, in addition to the possible interspecies differences in sensitivity to glyphosate, the apparent discrepancies between the results

Table 2
Retinoids, carotenoids and α-tocopherol contents measured in honey bees (ng/g tissue) exposed for 10 days to atrazine (ATZ, 5 ng/bee), glyphosate (GLY, 5 ng/bee) in the absence or the presence of 0.03 ppm Cd. Data are means ± SD estimated on 8 to 10 pools of two or three bees.

Sugar syrup	13-*cis*-4-oxo-RA	13-*cis*-RA	9-*cis*-RA	ROH	RALD	Zeaxanthin	α-carotene	β-carotene	α-tocopherol
Control	7.75 [a]	8.26	4.82[ab]	21.5[a]	27.6	42.6[ab]	45.7[a]	145[a]	206[a]
	(3.67)	(1.49)	(0.59)	(4.32)	(4.66)	(28.2)	(10.4)	(53.1)	(56.3)
ATZ	7.93 [a]	7.68	3.91[a]	20.9[a]	21.2	49.8[ab]	36.0[ab]	100[ab]	131[b]
	(2.02)	(2.76)	(0.74)	(4.91)	(1.86)	(32.6)	(5.7)	(26.8)	(22.5)
GLY	10.8 [a]	14.6	6.15[ab]	19.1[a]	19.3	43.5[ab]	45.2[a]	95.0[ab]	172[abc]
	(4.04)	(6.96)	(3.08)	(5.29)	(2.96)	(22.7)	(6.2)	(22.4)	(65.8)
Cd	10.9 [a]	10.9	4.25[a]	25.0[a]	29.5	37.7[ab]	38.9[ab]	105[ab]	196[ac]
	(10.3)	(11.75)	(1.66)	(4.37)	(3.09)	(20.5)	(4.8)	(23.2)	(56.5)
ATZ + Cd	8.61 [a]	11.4	4.99[ab]	19.2[a]	26.6	27.1[a]	46.9[a]	83.9[b]	176[abc]
	(3.93)	(8.81)	(1.35)	(3.26)	(4.10)	(6.74)	(9.9)	(19.4)	(46.9)
GLY + Cd	14.6 [a]	11.1	5.33[ab]	24.3[a]	28.9	65.0[b]	31.3[b]	115[b]	174[abc]
	(11.9)	(4.74)	(2.33)	(4.92)	(4.52)	(26.3)	(3.9)	(34.2)	(19.1)
ATZ + GLY	8.44 [a]	10.5	7.81[b]	23.7[a]	27.5	67.1[b]	32.9[b]	91.1[b]	194[abc]
	(2.22)	(2.15)	(3.26)	(2.69)	(3.55)	(31.8)	(3.8)	(14.1)	(100)
ATZ + GLY + Cd	41.4 [b]	15.1	4.52[a]	23.3[a]	29.5	44.1[ab]	32.8[b]	93.5[b]	214[abc]
	(29.2)	(12.3)	(1.78)	(5.60)	(5.99)	(17.2)	(5.3)	(21.6)	(93.5)
Statistic model	$KW_{7,74} = 20.9$ $p < 0.01$	$D_{7,63} = 1.00$ $p = 0.442$	$KW_{7,70} = 15.5$ $p < 0.05$	$D_{7,70} = 2.44$ $p < 0.05$	$D_{7,70} = 0.82$ $p = 0.574$	$KW_{7,77} = 15.0$ $p < 0.05$	$D_{7,60} = 8.38$ $p < 0.001$	$KW_{7,78} = 17.8$ $p < 0.05$	$KW_{7,78} = 19.2$ $p < 0.01$

D: GLM one-way analysis of variance followed by adjusted pairwise Bonferroni test ($p < 0.05$).
KW: Kruskal-Wallis non-parametric test followed by Dunn's test ($p < 0.05$).
Data not sharing the same letters are statistically different.

Table 3
Retinoids, carotenoids, α-tocopherol and protein contents measured in honey bees (ng/g tissues) exposed for $10^{(1)}$ or $5^{(2)}$ days to increasing concentrations of Fe in the absence or the presence of 0.03 ppm Cd. Data are means ± SD estimated on 7 to 10 pools of two or three bees.

Metal (mg/L sugar syrup)	13-*cis*-4-oxo-RA	13-*cis*-RA	9-*cis*-RA	ROH	RALD	Zeaxanthin	α-carotene	β-carotene	α-tocopherol
Control	12.2	9.77	9.32	27.7	33.7	22.7[a]	37.0	108	167[ab]
	(13.66)	(4.22)	(9.15)	(5.23)	(5.56)	(3.3)	(5.6)	(10.4)	(24.9)
Fe (40) [(1)]	6.11	8.81	4.96	27.5	30.6	12.8[b]	44.9	88.5	113[a]
	(1.96)	(3.06)	(2.95)	(10.0)	(7.86)	(7.8)	(9.7)	(16.5)	(32.7)
Fe (200) [(1)]	12.3	9.23	4.59	27.9	30.7	11.7[b]	42.0	92.5	110[a]
	(13.7)	(2.45)	(1.09)	(5.43)	(4.30)	(3.3)	(8.6)	(9.3)	(43.3)
Fe (800) [(2)]	11.5	10.75	6.32	22.7	30.4	14.6[ab]	43.9	97.9	177[b]
	(5.04)	(2.26)	(2.00)	(6.44)	(7.58)	(5.7)	(8.6)	(25.9)	(49.9)
Fe (40) + Cd [(1)]	8.52	10.10	6.87	27.9	30.9	16.6[ab]	37.9	96.2	154[ab]
	(6.96)	(3.21)	(3.13)	(7.01)	(4.85)	(6.3)	(9.5)	(28.8)	(51.1)
Fe (200) + Cd [(1)]	6.27	8.55	8.25	26.7	29.6	11.5[b]	43.7	82.1	148[ab]
	(1.54)	(2.22)	(3.71)	(8.64)	(6.77)	(3.8)	(7.8)	(22.9)	(36.9)
Fe (800) + Cd [(2)]	10.73	7.80	5.80	24.0	28.8	17.2[ab]	34.9	90.1	145[ab]
	(6.45)	(1.58)	(3.02)	(5.79)	(3.98)	(5.0)	(2.7)	(19.3)	(60.4)
Statistic model	$KW_{6,59} = 10.32$ $p = 0.112$	$D_{6,51} = 0.96$ $p = 0.46$	$KW_{6,58} = 8.82$ $p = 0.184$	$D_{6,54} = 0.76$ $p = 0.603$	$D_{6,54} = 0.54$ $p = 0.78$	$KW_{6,61} = 17.9$ $p < 0.01$	$D_{6,54} = 2.10$ $p = 0.07$	$D_{6,54} = 1.38$ $p = 0.24$	$D_{6,54} = 2.93$ $p < 0.05$

D: GLM one-way analysis of variance followed by adjusted pairwise Bonferroni test ($p < 0.05$).
KW: Kruskal-Wallis non-parametric test followed by Dunn's test ($p < 0.05$).
Data not sharing the same letters are statistically different.

obtained may underline different effects resulting from chronic *vs.* acute exposure. In contrast to the moderate effects of herbicides, metals induced much more pronounced variations in the TBARS levels. Increases of 55% and 87% were obtained with 40 ppm Fe in the absence and the presence of Cd, respectively, and values up to 1.5 times the control levels were estimated in bees fed with 200 ppm Fe (±Cd) (Fig. 3B). TBARS content seems to increase with increasing doses of Fe. Note that levels measured with 800 ppm Fe were for a 5-day exposure in bees suffering from 10% body mass loss. While we have previously shown that 0.03 ppm Cd did not increase TBARS levels under our experimental conditions (Hedrei Helmer et al., 2015), we now show that it does not potentiate the Fe-induced TBARS production. Iron is well known for its high potency to induce oxidative stress. As with mammals, bees have intracellular proteins that bind Fe and maintain the intracellular free Fe at very low levels to prevent oxidative damage to the cell (Hsu and Chan, 2011). Although the TBARS assay is not a highly specific method for measuring lipid peroxidation, our data show that atrazine and glyphosate at doses less than 1% of the LD_{50} values, as well as Fe at levels measured in field maize pollen, may induce oxidative stress in bees. Alpha-tocopherol is well known to protect against oxidative stress. However, none of the bees' treatments that increased the TBARS levels has modified the contents of α-tocopherol or other antioxidants (lutein, zeaxanthin, α- and β-cryptoxanthin).

4. Conclusion

The present study reveals that mixtures of atrazine, glyphosate and Cd at environmentally relevant levels that do not affect bee viability may modify RA metabolism at steps downstream from RALD formation. Mixtures of herbicides and Cd lower bee α- and β-carotene contents. Higher levels of RA isomers (9-*cis*-RA) and metabolites (13-*cis*-4-oxo-RA) were observed in bees with low carotenoids, whereas ROH contents were not modified. Bee zeaxanthin concentrations were highly sensitive to either Cd or Fe, whereas

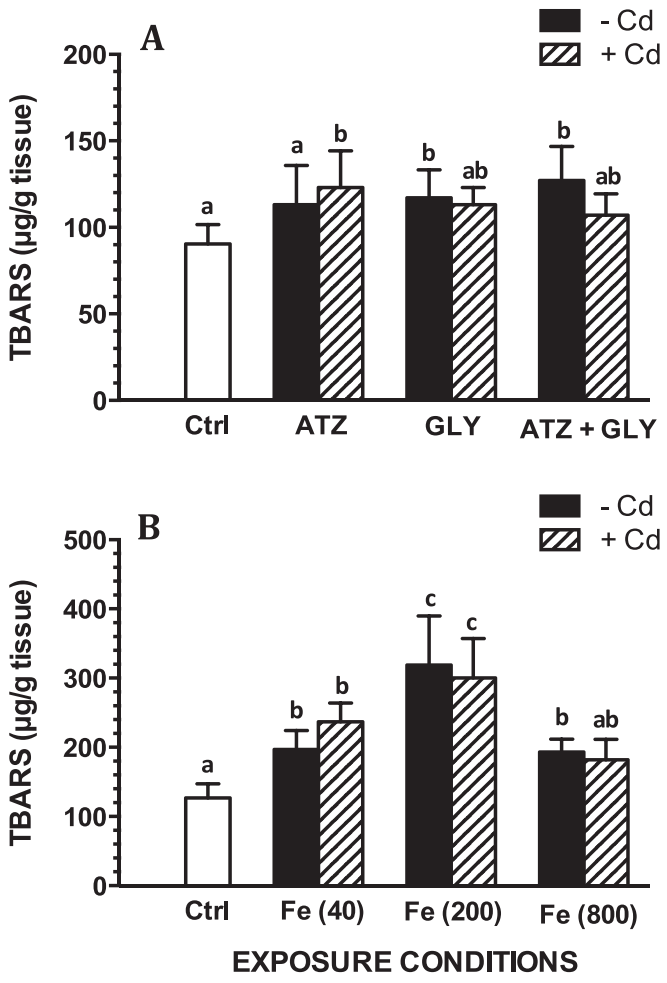

Fig. 3. TBARS (µg/g tissue) measured in bees exposed to: A) atrazine (ATZ, 5 ng/bee), glyphosate (GLY, 5 ng/bee) or a mixture of both in the absence (black columns) or the presence (dashed columns) of Cd (0.03 ppm); B) 40, 200 or 800 ppm Fe alone (black columns) or in combination with 0.03 ppm Cd (dashed columns). Exposure time was 10 days except for 800 ppm Fe (5 days). Data are means ± SD estimated on 9 to 10 pools of 8 bees. Groups were compared using: A) one-way analysis of variance followed by adjusted pairwaise Bonferroni tests, ($p < 0.001$); B) Kruskal-Wallis non parametric test followed by Dunn's test, ($p < 0.001$). Data with different letters are statistically different.

atrazine or glyphosate had no effect. Some mixtures of herbicides and Cd promoted lipid peroxidation. The data also show that orally ingested Fe at a concentration found in the maize pollen is available to the bee and is steadily accumulated over 10 days. Iron induced high levels of lipid peroxidation. The data also suggests that under some exposure conditions, ingested Fe and Cd may compete in the oral absorption process. This interaction between metals for the intestinal absorption deserves to be investigated in future studies. All these data reveal that mixtures of atrazine, glyphosate, Cd and Fe may affect different steps in the metabolic pathway of vitamin A. The present study represents a further step in understanding the possible effects of realistic environmental exposures to herbicide and metal mixtures on the bee health.

Acknowledgements

The authors are grateful to the Direction des matières dangereuses et des pesticides, Ministère du Développement Durable, de l'Environnement et de la Lutte contre les Changements Climatiques (MDDELCC) for the metal analysis. We thank Nathalie Dassylva from CEAEQ (MDDELCC) for her valuable cooperation. The authors would like to thank Maxime Gauthier for improving the HPLC methods to better detect RA isomers and metabolites, as well as Isabelle Lecavalier-Mondor for the retinoids, carotenoids, α-tocopherol and TBARS analyses. This study was supported by a grant from the Programme de Soutien à l'Innovation en Agro-alimentaire (PSIA) from Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ), awarded to M. Boily (#811175).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.10.056.

References

Aizen, M.A., Herder, L.D., 2009. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr. Biol. 19, 915–918. http://dx.doi.org/10.1016/j.cub.2009.03.071.

Armstrong, J.L., Redfern, C.P., Veal, G.J., 2005. 13-*cis* Retinoic acid and isomerisation in paediatric oncology. Is changing shape the key to success? Biochem. Pharmacol. 69, 1299–1306.

ARLA (Agence de réglementation de la lutte antiparasitaire), 2007. Décision de réévaluation: atrazine (Évaluation environnementale). Santé Canada, Sécurité des produits de consommation. RVD2007–05.

Bastien, J., Rochette-Egly, C., 2004. Nuclear retinoid receptors and the transcription of retinoid-target genes. Gene 328, 1–16. http://dx.doi.org/10.1016/j.gene.2003.12.005.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Boily, M., Sarrasin, B., DeBlois, C., Aras, P., Chagnon, M., 2013. Acetylcholinesterase in honey bees (*Apis mellifera*) exposed to neonicotinoids, atrazine and glyphosate: laboratory and field experiments. Environ. Sci. Pollut. Res. 20, 5603e5614. http://dx.doi.org/10.1007/s11356-013-1568-2.

Cellier, M., Privé, G., Belouchi, A., Kwan, T., Rodrigues, V., Chia, W., Gros, P., 1995. Nramp defines a family of membrane proteins. Proc. Natl. Acad. Sci. U. S. A. 92, 10089–10093.

Cui, Y., Freedman, J.H., 2009. Cadmium induces retinoic acid signaling by regulating retinoic acid metabolic gene expression. J. Biol. Chem. 284, 24925–24932. http://dx.doi.org/10.1074/jbc.M109.026609.

de Aguiar, L.M., Figueira, F.H., Gottschalk, M.S., da Rosa, C.E., 2016. Glyphosate-based herbicide exposure causes antioxidant defense response in the fruit fly *Drosophila melanogaster*. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 185–186, 94–101. http://dx.doi.org/10.1016/j.cbpc.2016.03.006. Epub 2016 Mar 12.

Di, N., Hladun, K.R., Zhang, K., Liu, T.X., Trumble, J.T., 2016. Laboratory bioassays on the impact of cadmium, copper and lead on the development and survival of honeybee (*Apis mellifera L.*) larvae and foragers. Chemosphere 152, 530–538.

Elisma, F., Jumarie, C., 2001. Evidence for cadmium uptake through Nramp2: metal speciation studies with Caco-2 cells. Biochem. Biophys. Res. Commun. 285, 662–668. http://dx.doi.org/10.1006/bbrc.2001.5245.

Folwell, J.L., Barton, C.H., Sheppard, D., 2006. Immunolocalisation of the *D. melanogaster Nramp* homologue *Malvolio* to gut and Malpighian tubules provides eveidence that *Malvolio* and *Narmp2* are orthologous. J. Exp. Biol. 209, 1988–1995.

Gauthier, M., Aras, P., Jumarie, C., Boily, M., 2016. Low dietary levels of Al, Pb and Cd may affect the non-enzymatic antioxidant capacity in caged honey bees (*Apis mellifera*). Chemosphere 144, 848–854.

Gorse, I., Rivard, L., 2011. Bilan des ventes de pesticides au Québec pour l'année 2008. Ministère du Développement durable, de l'Environnement et des Parcs, Québec. ISBN (PDF) 978-2-550-61586-6, 85 p.

Gunshin, H., Mackenzie, B., Bergler, U.V., Gunshin, Y., Romero, M.F., Boron, W.F., Nussberger, S., Gollan, J.L., Hediger, M.A., 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388, 482–488.

Hladun, K., Di, N., Liu, T.-X., Trumble, J.T., 2016. Metal contaminant accumulation in the hive: consequences for whole-colony health and brood production in the honey bee (*Apis mellifera L.*). Environ. Toxicol. Chem. 35, 322–329.

Hedrei Helmer, S., Kerbaol, A., Aras, P., Jumarie, C., Boily, M., 2015. Effects of realistic doses of atrazine, metolachlor, and glyphosate on lipid peroxidation and diet-derived antioxidants in caged honey bees (*Apis mellifera*). Environ. Sci. Pollut. Res. 22, 8010–8021. http://dx.doi.org/10.1007/s11356-014-2879-7.

Henson, T.M., Cory, W., Rutter, M.T., 2013. Extensive variation in cadmium tolerance and accumulation among populations of *Chamaecrista fasciculata*. PLoS One 8 (5), e63200. http://dx.doi.org/10.1371/journal.pone.0063200.

Hsu, C.Y., Chan, Y.P., 2011. Identification and localization of proteins associated with biomineralization in the iron deposition vesicles of honeybees (*Apis mellifera*). PLoS One 6 (4), e19088. http://dx.doi.org/10.1371/journal.pone.0019088.

ISQ (Institut de la statistique du Québec), MAPAQ (Ministère de l'Agriculture, des

Pêcheries et de l'Alimentation du Québec), 2013. Profil sectoriel de l'industrie bioalimentaire du Québec. édition 2013. Gouvernement du Québec. ISBN 978-2-550-70028-9 (PDF).
Jasper, R., Locatelli, G.O., Pilati, C., Locatelli, C., 2012. Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup®. Interdiscip. Toxicol. 5, 133–140.
Keshk, W.A., Soliman, N.A., Abo El-Noor, M.M., Wahdan, A.A., Shareef, M.M., 2014. Modulatory effect of curcumin on redox status, mitochondrial function, and caspase-3 expression during atrazin-induced toxicity. J. Biochem. Mol. Toxicol. 28, 378–385. http://dx.doi.org/10.1002/jbt.21574.
Krupke, C.H., Hunt, G.J., Eitzer, B.D., Andino, G., Given, K., 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One 7, e29268. http://dx.doi.org/10.1371/journal.pone.0029268.
Lambert, O., Piroux, M., Puyo, S., Thorin, C., Larhantec, M., Delbac, F., Pouliquen, H., 2012. Bees, honey and pollen as sentinels for lead environmental contamination. Environ. Pollut. 170, 254–259. http://dx.doi.org/10.1016/j.envpol.2012.07.012.
Lee, G.S., Liao, X., Cantor, R.M., Collins, M.D., 2006. Interactive effects of cadmium and all-*trans*-retinoic acid on the induction of forelimb ectrodactyly in C57BL/6 mice. Birth Defects Res. A Clin. Mol. Teratol. 76, 19–28.
Mărgăoan, R., Mărghitas, L.A., Dezmirean, D.S., Dulf, F.V., Buena, A., Socaci, S.A., Bobis, O., 2014. Predominant and secondary pollen botanical origins influence the carotenoïd and fatty acide profile in fresh honeybee-collected pollen. J. Agric. Food Chem. 62, 6306–6316.
McKenzie, R.M., Hellwege, D.M., McGregor, M.L., Nelson, E.C., 1979. Oxidation and isomerisation of retinoid acid by iodine and light: a novel preparation of all-*trans*- and 13-*cis*-4-oxoretinoic acid. Lipids 14, 714–717.
MDDEP (Ministère du Développement Durable, de l'Environnement et des Parcs), 2012. Guide sur le recyclage des matières résiduelles fertilisantes: critères de référence et normes réglementaires, p. 170.
Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., van Engelsdorp, D., Pettis, J.S., 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS One 5 (3), e9754. http://dx.doi.org/10.1371/journal.pone.0009754.
Nakamura, A., Stiebler, R., Fantappié, M.R., Fialho, E., Masuda, H., Oliveira, M.F., 2007. Effects of retinoids and juvenoids on moult and on phenoloxidase activity in the blood-sucking insect *Rhodnius prolixus*. Acta Trop. 103, 222–230.
Nwani, C.D., Lakra, W.S., Nagpure, N.S., Kumar, R., Kushwaha, B., Srivastava, S.K., 2010. Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa Punctatus* (Bloch). Int. J. Environ. Res. Public. Health 7, 3298–3312.
Oberhauser, V., Voolstra, O., Bangert, A., von Linting, J., Vogt, K., 2008. NinaB combines carotenoid oxygenase and retinoid isomerase activity in a single polypeptide. Proc. Natl. Acad. Sci. U. S. A. 105, 19000–19005. http://dx.doi.org/10.1073/pnas.0807805105.
OECD (Organisation of Economic Cooperation and Development), 1998. OECD Guidelines for the Testing of Chemicals: Honeybees, Acute Oral Toxicity Test, 8p.
Petkovitch, P.M., 2001. Retinoic acid metabolism. J. Am. Acad. Dermatol. 45, S136–S142.
Pettis, J.S., Lichtenberg, E.M., Adnree, M., Stizinger, J., Rose, R., vanEngelsdorp, D., 2013. Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae*. PLoS One 8 (7), e70182. http://dx.doi.org/10.1371/journal.pone.0070182.
Rodrigues, V., Cheah, P.Y., Ray, K., Chia, W., 1995. *malvolio*, the *Drosophila* homologue of mouse NRAMP-1 (*Bcg*), is expressed in macrophages and in the nervous system and is required for normal taste behavior. EMBO J. 14, 3007–3020.
Solari, M., Paquin, J., Ducharme, P., Boily, M., 2010. P19 neuronal differentiation and retinoic acid metabolism as criteria to investigate atrazine, nitrite, and nitrate developmental toxicity. Toxicol. Sci. 113, 116–126.
Thornton, B.J., Elthon, T.E., Cerny, R.L., Siegfried, B.D., 2010. Proteomic analysis of atrazine exposure in *Drosophila melanogaster* (Diptera: Drosophilidae). Chemosphere 81, 235–241.
Tsukada, M., Schröder, M., Roos, T.C., Chandraratna, R.A.S., Reichert, U., Merk, H.F., Orfanos, C.E., Zouboulis, C.C., 2000. 13-*cis* Retinoic acid exerts its specific activity on human sebocytes through selective intracellular isomerization to all-*trans* retinoic acid and binding to retinoic acid receptors. J. Investig. Dermatol. 115, 321–327.
Veal, G.J., Errington, J., Redfern, C.P., Pearson, A.D., Boddy, A.V., 2002. Influence of isomerisation on the growth inhibitory effects and cellular activity of 13-cis and all-trans retinoic acid in neuroblastoma cells. Biochem. Pharmacol. 63, 207–215.
von Lintig, J., Dreher, A., Kiefer, C., Wernet, M., Vogt, K., 2001. Analysis of the blind *Drosophila* mutant *ninaB* identifies the gene encoding the key enzyme for vitamin A formation *in vivo*. Proc. Natl. Acad. Sci. U. S. A. 98, 1130–1135. http://dx.doi.org/10.1073/pnas.031576398.
Wang, A., Wang, M., Lio, Q., He, X., 2016. Characterization of Cd translocation and accumulation in 19 maize cultivars grown on Cd-contaminated soil: implication of maize cultivar selection for minimal risk to human and for phytoremediation. Environ. Sci. Pollut. Res. Int. 23, 5410–5419. http://dx.doi.org/10.1007/s11356-015-5781-z.
Wu, J.Y., Anelli, C.M., Sheppard, W.S., 2011. Sub-lethal effects of pesticides residues in brood comb on worker honey bee (*Apis Mellifera*) development and longevity. PLoS One 6. http://dx.doi.org/10.1371/journal.pone.0014720.
Wu, X., Cobbina, S.J., Mao, G., Xu, H., Zhang, Z., Yang, L., 2016. A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. Environ. Sci. Pollut. Res. Int. 23, 8244–8259. http://dx.doi.org/10.1007/s11356-016-6333-x.
Xu, W., Lu, G., Dang, Z., Liao, C., Chen, Q., Yi, X., 2013. Uptake and distribution of Cd in sweet maize grown on contaminated soils: a field-scale study. Bioinorg. Chem. Appl. http://dx.doi.org/10.1155/2013/959764.