COMPARATIVE TOXICITY OF IMIDACLOPRID, OF ITS COMMERCIAL LIQUID FORMULATION AND OF DIAZINON TO A NON-TARGET ARTHROPOD, THE MICROCRUSTACEAN *Daphnia magna*

Anita Jemec [1], Tatjana Tišler [1], Damjana Drobne [2], Kristina Sepčić [2], Didier Fournier [3] Polonca Trebše [4] *

[1]National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

[2]University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia

[3]IPBS-UMR 5089, Institut de Pharmacologie et de Biologie Structurale, Group de Biophysique et de Biotechnologie des Proteines, 205 Route de Narbonne, 31077 Toulouse, France

[4]University of Nova Gorica, Laboratory for Environmental Research, P.O.B. 301, SI-5001 Nova Gorica, Slovenia

*CORRESPONDING AUTHOR:

POLONCA TREBŠE

University of Nova Gorica

Laboratory for Environmental Research

Vipavska 13, P.O.B. 301, SI-5001 Nova Gorica, Slovenia, Europe

PHONE: +386 5 3315 238

FAX: +386 5 3315 296; E- MAIL: polonca.trebse@p-ng.si

Abstract

Imidacloprid (IMI) is at the moment the insecticide with the world's fastest growing sales and is considered possible replacement for the widely used organophosphorus pesticide, diazinon, which is subject to phased revocation in many countries. In this study, biochemical, reproductive and survival parameters of the water flea (*Daphnia magna*) after chronic exposure to IMI, its commercial liquid formulation Confidor SL 200 and diazinon are presented and compared. According to the lowest observed effect concentrations, diazinon is more toxic to the reproduction of *D. magna* than IMI and Confidor SL 200, which exert similar toxicity. The same was observed for the survival, except that Confidor SL 200 is more toxic than IMI. In polluted aquatic environments, the actual levels of diazinon are potentially chronically hazardous to the reproduction of *D. magna* (risk quotient >1). According to very few measured environmental levels of IMI, the latter is not expected to be chronically hazardous, unless it is accidentally spilled in a small pond. In such case, the predicted concentrations of IMI would present a potential chronic risk to *D. magna*, and a potential acute risk to other aquatic invertebrates. In the future, higher environmental levels of IMI are expected due to its increasing use and physico-chemical properties. The literature survey summarized in this work suggests that further ecotoxicological studies with a broader spectrum of aquatic organisms are needed before IMI is classified as safer than currently applied pesticides.

Keywords: Confidor SL 200, cholinesterase, catalase, glutathione S-transferase

1. Introduction

The insecticide imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine] (IMI) has been increasingly used since 1991 (Elbert et al., 1991) and belongs to the fastest growing group of insecticides introduced to the market, referred to as neonicotinoids (Tomizawa and Casida, 2003). It acts as an agonist of the postsynaptic nicotinic acetylcholine receptors (Matsuda et al., 2001), disrupting the normal neural processes, and is used mainly to control sucking insects on crops (Tomlin, 1997; Tomizawa and Casida, 2005). IMI is a potential groundwater and surface water contaminant (PAN Pesticides database, 2006), because it can leach and runoff from soil and crops (Felsot et al., 1998; Gonzalez-Pradas et al., 1999; Armbrust and Peeler, 2002; Gupta et al., 2002; Fossen, 2006). Additionally, it may enter water bodies from spray drift or accidental spills, leading to local point-source contaminations.

IMI is considered a possible replacement for urban uses of diazinon (TDC Environmental, 2003), one of the most used insecticides in the last 50 years. Namely, diazinon is currently subject to phased revocation in USA (U.S. EPA, 2004), European Union and Australia (APVMA, 2003), because unacceptable risk to agricultural workers and environment was proved. As a result, the annual use of diazinon has already declined, for instance in USA (California) by 65% in the years 1994-2004 (California DPR, 2004).

To regulate the impacts of IMI on aquatic ecosystems, its toxicological profile needs to be thoroughly established. Until now, the toxicity of IMI to aquatic invertebrates has rarely been assessed and very few monitoring studies of this insecticide have been performed in aquatic environments (Table 1). This is due to the former belief that the compound is relatively immobile in soil and does not leach to groundwater (Bayer technical information for Confidor®, 2000; Krohn and Hellpointer, 2002).

A variety of standard toxicity tests are available for testing the toxicity of chemicals present in aquatic environment. Standard acute (ISO 6341:1996) and chronic (ISO 10706: 2000) toxicity test with the water flea *Daphnia magna* are among the most used, where immobility and reproduction are monitored, respectively. In the case of low concentrations of chemicals, biochemical biomarkers are generally considered a more sensitive and sometimes more specific measure of toxic exposure and effect than the survival, however this approach is not standardised yet (Adams, 2002).

Among the most commonly analyzed biochemical biomarkers are the activities of cholinesterases (ChE), glutathione S-transferase (GST) and catalase (CAT). The inhibition of ChE by organophosphorus and carbamate pesticides results in overaccumulation of the neurotransmitter and, as a consequence, prolonged electrical activity at nerve endings (Chambers, 1992). GST catalyses the conjugation of glutathione with xenobiotics, including organophosphorus pesticides (Booth and O'Halloran, 2001), and the cytotoxic aldehydes produced during lipid peroxidation (Halliwell and Gutteridge, 1999). Catalase decomposes the hydrogen peroxide extensively formed during oxidative stress (Halliwell and Gutteridge, 1999). Protein content in *D. magna* is also used as a biomarker of chronic chemical exposure (Knowles and Mckee, 1987), and reflects the entire physiological state of the organism (Printes and Callaghan, 2003).

In this study, chronic effects of IMI on different biochemical, reproductive, and survival parameters of *D. magna* were determined. Chronic effects of IMI on *D. magna* have rarely been evaluated; only one publicly inaccessible study describing the effects of IMI on the reproduction of *D. magna* (Young and Blakemore, 1990) has been conducted so far. The hazards of chemicals were compared using risk quotients (RQ); e.g. the ratio between the estimated/detected environmental concentrations divided by chronic toxicity values (21 d LOEC; the lowest observed exposure concentration that produces a statistically different

response from the control response after 21 d) (U.S. EPA, 2004). The chemical was considered potentially chronically hazardous if RQ>1, and acutely hazardous when RQ>0.5. Higher RQ value corresponds to the higher potential risk (U.S. EPA, 2004). The toxicity data of IMI were compared with its commercial liquid formulation (Confidor SL 200; containing 200 g/L of IMI in solvents) and with diazinon.

The aims of this work were: (1) to assess the chronic effects of IMI on biochemical, reproductive, and survival parameters in a non-target arthropod, *D. magna,* and (2) to compare its effects with its commercial liquid formulation Confidor SL 200 and with the organophosphorus pesticide diazinon. The comprehensive literature data on physico-chemical properties and environmental fate of IMI and diazinon and their toxicities to aquatic organisms are provided. The environmental risks of IMI and diazinon based on the actual and expected environmental concentrations are discussed.

2. Materials and Methods

2.1 *Chemicals*

The following chemicals were purchased from Sigma (Germany): dibasic and monobasic potassium phosphate, hydrogen peroxide (30%), 1-chloro-2,4-dinitrobenzene, L-glutathione (reduced form), 5,5-dithiobis-2-nitrobenzoic acid, sodium hydrogen carbonate, acetylthiocholine chloride, and ethylenediaminetetraacetic acid. BCA Protein Assay Reagents A and B were purchased from Pierce (U.S.A.). Diazinon and 1-methyl-2-pyrrolidone were provided by Pestanal, Riedel-de Haën (Seelze, Germany); imidacloprid, Confidor SL 200 by Bayer CropScience AG (Monheim, Germany), and dimethylsulfoxide by Merck (Darmstadt,

98 Germany). All chemicals were of the highest commercially available grade, typically 99% or
99 higher.
100

101 2.2 *Chronic toxicity test with* Daphnia magna *Straus 1820 (water flea)*

102

103 Water fleas (*Daphnia magna* Straus 1820) were obtained from the Institut für Wasser,
104 Boden und Lufthygiene des Umweltbundesamtes (Berlin, Germany). They were cultured in
105 2.5 L of modified M4 media (Kühn et al., 1989) at 21±1 °C and 16:8 h light/dark regime
106 (1800 lux) with a diet of the algae *Desmodesmus subspicatus* Chodat 1926 corresponding to
107 0.13 mg carbon/daphnia per day.
108 Our laboratory is accredited according to ISO 17025:1999 for standard acute testing
109 with *D. magna*. Chronic toxicity to daphnids was evaluated using a semi-static exposure
110 system under the same conditions as culturing (ISO 10706: 2000). Individual daphnids less
111 than 24 h old were placed in 50 mL of test solution; 10 test containers per each concentration
112 and a control were prepared. Chronic tests for each chemical were repeated up to three times.
113 The survived initial daphnids were transferred into freshly prepared test solutions three times
114 per week. The animals were fed daily a diet of *Desmodesmus subspicatus* (0.13 mg
115 carbon/daphnia per day) and the newly born neonates were counted and removed. The criteria
116 used to evaluate reproduction after 21 d were the number of neonates per adult, the average
117 brood size per adult, the number of broods per adult, and the time to the first reproduction.
118 The mortality of the daphnids during 21 d was also monitored.
119 The following concentrations of IMI: 0, 0.625, 1.25, 2.5, 5, 10, 20, 40 mg L^{-1} and
120 diazinon: 0, 0.0753, 0.165, 0.312, 0.625, 1.25, 2.5, 5, 8 μg L^{-1} were tested. Confidor SL 200
121 was diluted in distilled water to obtain the following solutions: 0, 0.000625, 0.00125, 0.0025,

0.005, 0.01, 0.02% (v/v); which contained 0, 1.25, 2.5, 5, 10, 20, 40 mg L^{-1} of IMI, respectively. The toxicity of solvents incorporated in Confidor SL 200 (a solution consisting of 38.4% of dimethylsulfoxide, 37.5% of 1-methyl-2-pyrrolidone and 24.1% of distilled water in place of IMI) was tested to exclude the possible toxic effect. The concentration of this negative control was equivalent to the highest concentration of Confidor SL 200 used in the tests (0.02%; v/v).

2.3 *Monitoring of the stability of test chemicals during the tests*

The test media were changed every two days. Prior to toxicity tests, the stabilities of IMI and diazinon in the test solution were checked. The test solutions were exposed separately to the same experimental conditions as the toxicity tests and the concentrations of the specific chemicals were measured at the outset and after two days.

Diazinon solution (10 mL) was extracted with three portions of ethyl acetate (25, 20 and 10 mL) with the addition of 50 mL of a 10% aqueous solution of sodium hydrogen carbonate (Bavcon et al., 2003). The solvent was evaporated, and the residue redissolved in 1 mL of ethyl acetate and analysed by gas chromatography (HP 6890, Germany)) with a flame ionization detector. Extraction of IMI was performed on initially preconditioned Strata C18-E columns (Phenomenex, USA) with 5 mL of methanol and 5 mL of distilled water (Baskaran et al., 1997). 1 mL of IMI solution was added to the column, and afterwards eluted with 2 mL of methanol. The solvent was evaporated and dried IMI was dissolved in 1 mL of acetonitrile-water (20:80, v/v) solution. The samples were analyzed on Agilent 100 Series liquid chromatograph (Germany) equipped with DAD detector on Zorbax C8 column.

145 Our experiments showed no changes in concentrations of IMI and diazinon in test
146 solutions during two days of exposure to the same experimental conditions as in the toxicity
147 tests. No degradation products of diazinon were detected. The actual exposure concentrations
148 of both chemicals did not differ by more than 20% from the nominal or initial concentrations.
149 Therefore the results are given in nominal concentrations, as suggested by ISO 10706: 2000.
150

151 2.4 *Determination of enzyme activities*

152

153 For each experiment, 10 test containers per each concentration of the chemical were
154 prepared. After 21 d in presence of the chemicals, five adult daphnids per concentration were
155 combined into one enzyme sample, thus two samples were prepared for each concentration.
156 Since each experiment was repeated three times, a total of six samples per concentration were
157 prepared. Prior to homogenization, excess chemical was removed from the surface of the
158 animals by rinsing three times with 2 mL of 50 mM phosphate buffer pH 7.0 combined with 5
159 mM ethylenediaminetetraacetic acid (Jemec et al., 2007). The animals were then
160 homogenized for 3 min in 0.8 mL of 50 mM phosphate buffer pH 7.0, using a glass–glass
161 Elvehjem-Potter homogenizer. The homogenate was centrifuged for 25 min at 15000 *g* and 4
162 °C. Enzyme activities were measured on freshly prepared supernatants.
163 ChE activity was determined according to Ellman et al. (1961), using microtiter plates
164 (Bio-Tek® Instruments, USA; PowerWave™ XS) as described by Jemec et al. (2007). The
165 reaction mixture was prepared in 100 mM of potassium phosphate buffer pH 7.3 containing
166 acetylthiocholine chloride and 5,5' dithiobis-2-nitrobenzoic acid in the final concentrations of
167 1 mM and 0.5 mM, respectively. 100 µL of protein supernatant were added to start the
168 reaction, which was followed spectrophotometrically at 412 nm and 25 °C for 15 min.

GST activity was measured on microtiter plates (Bio-Tek® Instruments, USA; PowerWave™ XS) (Habig et al., 1974; Jemec et al., 2007). 1-chloro-2,4-dinitrobenzene was dissolved in ethanol to obtain a 50 mM solution, which was afterwards diluted with 100 mM potassium phosphate buffer pH 6.5 to the final concentration of 4 mM. This solution was used to prepare a reaction mixture containing 1 mM of 1-chloro-2,4-dinitrobenzene and 1 mM of reduced glutathione. 50 µL of protein supernatant were added to start the reaction, which was followed spectrophotometrically at 340 nm and 25 °C for 3 min.

CAT activity was determined according to Aebi (1984). 50 µL of protein supernatant were combined with 750 µL of hydrogen peroxide solution (10.8 mM) prepared in 50 mM potassium phosphate buffer pH 7.0. The final concentration of hydrogen peroxide was 10 mM. The reaction was followed spectrophotometrically for 3 min at 25 °C and 240 nm on a Shimadzu UV-2101PC spectrophotometer (Japan).

Protein concentration was measured using a BCA™ Protein Assay Kit, a modification of the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA).

2.5 *Interpretation of enzyme activities*

Enzyme activities were expressed in enzyme units (EU) per one adult daphnia. Specific enzyme activities with protein content as a standard reference were also calculated for purposes of comparison. One EU was determined as the amount of ChE that hydrolyses 0.01 nmoles of acetylthiocholine min^{-1} (ε_{412} = 13600 $M^{-1}cm^{-1}$), the amount of CAT that degrades 1 µmole of hydrogen peroxide min^{-1} (ε_{240} = 43.6 $M^{-1}cm^{-1}$), and the amount of GST that conjugates 1 nmole of reduced glutathione min^{-1} (ε_{340} = 9600 $M^{-1}cm^{-1}$). These enzyme

192 units were chosen to facilitate the graphical comparison of all enzyme activities for each
193 chemical.
194

195 2.6 *Data analysis*

196

197 The 21 d LOEC values (e.g. the lowest observed effect concentration that produces a
198 statistically different response from the control response after 21 d) were determined by One-
199 way Analysis Of Variance (ANOVA; $P<0.05$), and the Games-Howell post hoc test for
200 biochemical parameters and Dunnett's test for reproduction data, using SPSS for Windows
201 8.0 (SPSS Inc., USA). The LOLC value for mortality was determined as the lowest observed
202 lethal concentration that causes mortality higher than 20% as allowed for control organisms
203 by the ISO standard (ISO 10706: 2000). The results for IMI and Confidor SL 200 were fitted
204 to sigmoid curves to calculate the slopes using the GOSA Software (www.bio-log.biz,
205 France). The values for unexposed control animals were not included in the data fitting, but
206 they are shown on graphs for comparison.
207

208 2.7 *Calculation of risk quotients (RQ) of tested chemicals*

209

210 Risk quotients (RQ) for all tested chemicals were calculated as a ratio between the
211 estimated/detected environmental concentrations divided by the LOEC for biochemical
212 parameters and reproduction, and LOLC for survival determined in this study. For the
213 comparison of the hazards of diazinon and IMI to different species of freshwater invertebrates
214 and vertebrates, RQ were calculated using LC_{50} (96 h) values based on literature data.

215 Only four monitoring studies are at the moment available on environmental levels of
216 IMI (Table 1). To determine RQ values for IMI, the lowest (1 μg L^{-1}), and the highest (14 μg
217 L^{-1}) measured values, estimated chronic value in surface waters (17.24 μg L^{-1}), and estimated
218 worse-case scenario level of accidental spill in a small pond (7300 μg L^{-1}) were used (Table
219 1). On the other hand, diazinon has been extensively monitored. The lowest (0.775 μg L^{-1}),
220 and the highest (24.6 μg L^{-1}) recently reported values in the literature, and the estimated value
221 in surface waters (429 μg L^{-1}) were used for calculation (Table 1).

222

223 **3. Results**

224

225 3.1 *Chronic toxicity tests*

226 In standard chronic toxicity tests with *D. magna*, reproduction and mortality of adult
227 daphnids were assessed. These data for all chemicals are shown in Table 2, columns 2-6. The
228 negative control (solvent mixture commercially used for the preparation of Confidor SL 200)
229 did not have any adverse effects on *D. magna* at the highest tested concentration of this
230 chemical (0.02%; v/v).

231 Tested concentrations of IMI and Confidor SL 200 have similar impacts on the
232 reproduction of *D. magna* (21 d LOEC = 2.5-10 mg L^{-1} for different reproduction parameters),
233 but Confidor SL 200 (21 d LOLC = 10 mg L^{-1} of IMI) affected their survival at lower
234 concentrations than IMI (21 d LOLC = 40 mg L^{-1}) (Table 2, Fig. 1).

235 Up to 5 μg L^{-1} of diazinon, the reproduction of daphnids was not affected. At this
236 concentration the mortality was 20%. At the next tested concentration of diazinon (8 μg L^{-1}),
237 the 100% mortality of daphnids was observed (Table 2).

238

3.2 *Enzyme activities*

In this study, the results of enzyme activities are expressed per animal and not per protein content, since the changes in protein content were observed as a result of exposure to the chemicals. The activities of all analyzed enzymes and the protein content in animals exposed to increasing concentrations of IMI and Confidor SL 200 decreased significantly (Fig.2a and 2b, Table 2).

In the experiments with diazinon, protein content of daphnids, ChE and GST activities did not change at any of the concentrations tested (up to 5 μg L^{-1}). Contrary to other analysed enzymes, CAT activities significantly decreased at 0.312, 0.625 and 1.25 μg L^{-1} of diazinon, but not at the highest concentrations 2.5 and 5 μg L^{-1} (Fig. 2c, Table 2).

To point out the importance of careful interpretation of enzyme activities, in case the protein content is changed during the exposure, specific enzyme activities per protein content were also calculated. In this case, CAT, GST and ChE activities increased significantly when exposed to IMI and Confidor SL 200. In the case of diazinon, enzyme activities were the same when calculated per animal or per protein content, since the protein content in this case did not change (not shown).

3.3 *Risk quotients of tested chemicals*

RQ values were calculated on the basis of recently detected and predicted aquatic levels of the chemicals tested, and on chronic toxicity data on *D. magna* gained in this work. These data show that only actual measured environmental levels of diazinon have RQ values higher than one, indicating them as potentially chronically hazardous to the reproduction of

263 *D. magna* (Table 3), while RQ values for Confidor SL 200 and IMI are lower than one. In the
264 case of an accidental spill, estimated concentrations of IMI and Confidor SL 200 would pose
265 a serious chronic risk to the reproduction and selected enzyme activities of *D. magna* (RQ>1).
266 Based on recent literature data, diazinon has higher RQ values for aquatic organisms
267 than IMI, but in general both chemicals are more harmful to aquatic invertebrates than fish
268 (Table 4). Actual measurements of diazinon levels in the environment show that this
269 insecticide is more hazardous to aquatic invertebrates (the highest calculated RQ = 117) than
270 IMI (the highest calculated RQ = 1.4). However, the risk of estimated concentrations of IMI
271 to aquatic invertebrates in the case of an accidental spill (the highest calculated RQ = 695.2)
272 is very high (Table 4).
273
274 **4. Discussion**
275
276 In this study, chronic effects of imidacloprid, its commercial liquid formulation
277 Confidor SL 200 and the organophosphorus pesticide diazinon on different biochemical,
278 reproductive, and survival parameters in *D. magna* were assessed and compared.
279 Enzyme activities were expressed per animal and not per protein amount, because
280 significant changes of the latter were found in daphnids exposed to IMI and Confidor SL 200.
281 This suggests that increasing concentrations of these chemicals affected not only the
282 investigated enzymes, but proteins in general. Consequently, enzyme activities expressed per
283 protein content differ from those expressed per animal, implying that cautious interpretation
284 of enzyme activities is needed in toxicity experiments. Similar point was raised by Printes and
285 Callaghan (2003).
286 The activities of ChE, GST and CAT in control adult daphnids (22 d old) expressed
287 per protein content were: 0.61 ± 0.043 nmol min^{-1} mg^{-1} protein; 87.26 ± 6.67 nmol min^{-1} mg^{-1}

288 protein and 84.28 ± 4.84 μmol min^{-1} mg^{-1} protein, respectively. These values are lower than
289 those previously recorded in juvenile daphnids (2.5-62.3 nmol min^{-1} mg^{-1} protein for ChE
290 (Guilhermino et al., 1996, Diamantino et al., 2000; Barata et al., 2001), 250 nmol min^{-1} mg^{-1}
291 protein for GST (Barata et al., 2005), and 250 μmol min^{-1} mg^{-1} protein for CAT (Barata et al.,
292 2005)). This is in agreement with Printes and Callaghan (2003) who observed significantly
293 lower ChE activity in 14-21 d old daphnids (0.5 nmol min^{-1} mg^{-1} protein) compared to 1-2 d
294 old juveniles (2.5 nmol min^{-1} mg^{-1} protein), and with our previous study (Jemec et al., 2007),
295 where the activities of juvenile daphnids were significantly higher than the values published
296 here for the adult ones. This apparent inverse relationship between the age and enzyme
297 activity was related to an increase in total protein of the animals during aging, which is not
298 proportional to the increase in the rate of substrate hydrolysis (Printes and Callaghan, 2003).
299 Our results indicate that tested concentrations of IMI and Confidor SL 200 have
300 similar impacts on the reproduction of *D. magna*, but Confidor SL 200 affected survival at
301 lower concentrations than IMI, possibly due to the synergism between the solvents and IMI.
302 The same was noticed for biochemical parameters, where Confidor SL 200 was slightly more
303 toxic than IMI (Table 2). The LOECs (2.5 mg L^{-1}) for the number of neonates per adult
304 exposed to IMI are similar to those reported by Young and Blakemore (1990), who found the
305 LOEC for reproduction at 7.3 mg L^{-1}.
306 The activities of all enzymes exposed to increasing concentrations of IMI and
307 Confidor SL 200 were significantly decreased in this study. No data on the chronic effect of
308 IMI on ChE, GST and CAT activities in daphnids are available in the literature. Only one
309 study by Capowiez et al. (2003) showed no acute effects on ChE and GST activities in
310 earthworms exposed up to 1 mg L^{-1} of IMI. The sensitivities of enzymes and reproduction
311 end-points of animals exposed to IMI in our study are similar (e.g. similar LOEC). This

312 suggests that the decrease of enzyme activities in this case is probably not an early, sensitive
313 biomarker of stress, but reflects a generally impaired physiological state of an organism.
314 In animals exposed to diazinon, no effects on the reproduction and survival of
315 daphnids up to 5 μg L^{-1} of diazinon were observed. However, already at 8 μg L^{-1}, 100%
316 mortality was determined. Published data on the LOEC values for the reproduction of *D.*
317 *magna* exposed to diazinon are very inconsistent. Fernandez-Casalderrey et al. (1995)
318 reported LOEC values for the reproduction in the range of 0.15-0.25 μg L^{-1}, while Sanchez et
319 al. (1998) found significantly lower LOEC values for the same endpoint performed in similar
320 experimental setup (0.00005 - 0.0005 μg L^{-1} of diazinon). Our higher LOEC values for the
321 reproduction of daphnids might be explained by differences in daphnid clones, and
322 experimental setup.
323 ChE activity was reported to be inhibited in daphnids exposed to organophosphates
324 (Day and Scott, 1990; Gälli et al., 1994), but no study has been performed on the effects of
325 organophosphorus pesticide diazinon on ChE activity in *D. magna* yet. Inhibition of ChE
326 activity was found in other organisms exposed to diazinon, for example in the white shrimp
327 *Litopenaeus vannamei* exposed to 12 μg L^{-1} of diazinon for 7 d (Gallindo-Reyes et al., 2000),
328 earthworm *Aporrectodea caliginosa* exposed to diazinon at 12 mg kg^{-1} (dry weight of soil)
329 (Booth and O'Halloran, 2001) and isopod *Porcellio scaber* at 5 μg g^{-1} of leaf (Stanek et al.,
330 2006). However, this paper and our previous work (Jemec et al., 2007) indicate that ChE
331 activity does not change in daphnids acutely and chronically exposed up to 5 μg L^{-1} of
332 diazinon. The differences in the changes of ChE activity after diazinon exposure can be
333 explained by species-specific biotransformation and detoxification mechanisms of diazinon to
334 a more potent diazoxon (Keizer et al., 1995). The induction of GST activity in diazinon-
335 treated organisms was expected, because GST is able to detoxify this insecticide (Chambers,
336 1992), but in this study no GST induction was detected when animals were exposed up to 5

337 μg L^{-1} of diazinon. The same observation was reported in our previous paper, where no
338 changes of GST activity were observed in daphnids acutely exposed up to 7 μg L^{-1} of
339 diazinon (Jemec et al., 2007). No other studies on chronic effects of diazinon on GST activity
340 in daphnids have previously been published.

341 There are very few data on environmental levels of IMI (only four studies in USA),
342 due to its irregular monitoring in aquatic environment. Based on our results, the levels of IMI
343 in freshwaters that have been detected so far (1- 14 μg L^{-1}), are not expected to be chronically
344 hazardous to the reproduction and survival of *D. magna* (RQ<1), however the same data are
345 reported to pose potential acute risk to some other aquatic invertebrates (RQ = 1.4). In
346 comparison to diazinon, actual aquatic levels of IMI are less hazardous (higher RQ) to aquatic
347 invertebrates, thus IMI is considered a possible replacement for diazinon (U.S. EPA, 2004).
348 However, in the case of accidental spill, estimated concentrations of IMI can also pose a
349 potential chronic risk to the reproduction of *D. magna* (RQ = 3), and acute risk to other
350 aquatic invertebrates (the highest calculated RQ = 695.2). Additionally, due to the increasing
351 use of IMI, one might expect significantly higher aquatic levels in the future. IMI also has
352 more physico-chemical properties that would favour its appearance in surface waters when
353 compared to diazinon (Table 1). It has higher water solubility, lower octanol-water partition
354 coefficient (K_{oc}), lower potential for sorption on soil (K_{ow}), and is more stable to hydrolysis
355 and soil degradation. Due to these characteristics, IMI is quite mobile in the environment and
356 stable on application sites, and it is very likely to be washed off the application sites,
357 especially off impervious surfaces (Oi, 1999). It degrades relatively quickly by aqueous
358 photolysis, but such decomposition can only occur at the surface of well-sunlit waters (TDC
359 Environmental, 2003; Fossen, 2006).

360 The toxicity of IMI is supposed to be very highly specific towards insects in
361 comparison to mammals, due to specific binding to the postsynaptic nicotinic acetylcholine

362 receptors (nAChR) of insects (Tomizawa and Casida, 2003). However, limited attention was
363 paid to binding affinity of IMI to the nAChRs of other arthropods or more generally
364 invertebrates. Additionally, the toxicity is not solely the result of binding between the ligand
365 and the receptor, but depends on many activities in the organism, such as the metabolism of
366 the chemical or its interactions with cell components. It was shown that the toxicity of IMI
367 towards aquatic invertebrates varies, with *D. magna* being less sensitive than others, for
368 instance amphipod *Hyalella azteca* or midge *Chironomus tentans,* and having acute LC_{50}
369 values in the same concentration range as fish (Table 1, Table 4). This suggests that the
370 toxicity of IMI is species-specific and may not easily be extrapolated to other organisms.
371 Relevant toxicity data could be obtained only when toxicity is tested with organisms
372 belonging to different taxonomic groups and trophic levels.
373
374 **In conclusion**, according to LOEC values, diazinon is more toxic to the reproduction
375 of *D. magna* than IMI and Confidor SL 200, which show similar toxicity. The same was
376 observed for the survival, except that commercial formulation (Confidor SL 200) is more
377 toxic than pure grade IMI. The actual aquatic levels of diazinon are potentially chronically
378 hazardous to the reproduction of *D. magna* (RQ>1), while recently detected concentrations of
379 IMI are not. Higher environmental levels of IMI are expected in the future due to its
380 increasing application and higher risk to aquatic organisms is anticipated. Additionally, we
381 have shown that in case IMI was accidentally spilled in a small pond, its predicted
382 environmental concentrations would chronically affect less sensitive organisms like *D. magna*
383 and acutely affect other, more sensitive aquatic invertebrates. Toxicity data on IMI presented
384 so far indicate that IMI is highly species-specific, therefore further (eco)toxicological studies
385 have to be performed with organisms belonging to different taxonomic groups, trophic levels
386 and habitats.

Acknowledgements

This work was financially supported by Slovenian Research Agency (projects no. J1-6001-1540-04, P2-0150). We thank Dr. Bill Milne for valuable comments and suggestions.

References

Abdel-Halim, K.Y., Salama, A.K., El-khateeb, E.N., Bakry, N.M., 2006. Organophosphorous pollutants (OPP) in aquatic environment at Damietta Governorate, Egypt: Implications for monitoring and biomarkers reponses. Chemosphere 63, 1491-1498.

Adams, S.M., 2002. Biological indicators of aquatic ecosystem stress. American Fisheries Society, Bethesda, Maryland, USA, pp. 1-23.

Aebi, H., 1984. Catalase *in Vitro*. Methods Enzymol. 105, 121- 126.

Anderson, T.D., Lydy, M.J., 2002. Increased toxicity to invertebrates associated with a mixture of atrazine and organophosphate insecticides. Environ. Toxicol. Chem. 21, 1507-1514.

Anderson, B.S., Hunt, J.W., Phillips, B.M., Nicely, P.A., de Vlaming, V., Connor, V., Richard, N., Tjeerdema, R.S., 2003. Integrated assessment of the impacts of agricultural drainwater in the Salinas River (California, USA). Environ. Pollut. 124, 523-532.

Ankley, G.T., Collyard, S.A., 1995. Influence of piperonyl butoxide on the toxicity of organophosphate insecticides to three species of freshwater bentic invertebrates. Comp. Biochem. Physiol. 110 C, 149-155.

APVMA (Australian Pesticides & Veterinary Medicines Authority), 2003. The reconsideration of registrations of products containing diazinon and their labels, Part 1: product cancellations.

412 *http://www.apvma.gov.au/chemrev/diazinon_reconsideration_part1_2003.pdf*
413 Armbrust, K.L., Peeler, H.B., 2002. Effects of formulation on the run-off of imidacloprid
414 from turf. Pest Manag. Sci. 58, 702-706.
415 Barata, C., Baird, D.J., Soares, A.M.V.M., Guilhermino, L., 2001. Biochemical factors
416 contributing to response variation among resistant and sensitive clones of *Daphnia magna*
417 Straus exposed to ethyl parathion. Ecotoxicol. Environ. Saf. 49, 155-163.
418 Barata, C., Varo, I., Navarro, J.C., Arun, S., Porte, C., 2005. Antioxidant enzyme activities
419 and lipid peroxidation in the freshwater cladoceran *Daphnia magna* exposed to redox
420 cycling compounds. Comp. Biochem. Physiol. 140 C, 175-186.
421 Baskaran, S., Kookana, R. S., Naidu, R., 1997. Determination of the insecticide imidacloprid
422 in water and soil using high-performance liquid chromatography. J. Chromatogr. A 787,
423 271-275.
424 Bavcon, M., Trebše, P., Zupančič-Kralj, L., 2003. Investigations of the determination and
425 transformations of diazinon and malathion under environmental conditions using gas
426 chromatography coupled with a flame ionisation detector. Chemosphere 50, 595-601.
427 Bayer technical information, Confidor®, 2000. Bayer, Germany.
428 Booth, L.H., O' Halloran, K., 2001. A comparison of biomarker responses in the earthworm
429 *Aporrectodea caliginosa* to the organophosphorous insecticides diazinon and chlorpyrifos.
430 Environ. Toxicol. Chem. 20, 2494-2502.
431 California DPR, 2004. California Department of Pesticide Regulation.
432 *http://www.cdpr.ca.gov/*
433 Capowiez, Y., Rault, M., Mazzia, C., Belzunces, L., 2003. Earthworm behaviour as a
434 biomarker- a case study using imidacloprid. Pedobiologia 47, 542-547.

435 Chambers, H.W., 1992. Organophosphorous compounds: An overview. In: Chambers, J.E.,
436 Levi, P.E., (Eds.), Organophosphates: chemistry, fate and effects. San Diego Academic
437 Press, USA, pp. 3-73.
438 Cobb, G.P., Mellott, R., Brewer, W., Bens, C.M., Kendall, R.J., 2000. Diazinon dissipation
439 from vegetation, occurrence in earthworms, and presence in avian gastrointestinal tracts
440 collected from apple orchards following D-Z-N ® 50W application. Environ. Toxicol.
441 Chem. 19, 1360-1367.
442 Cooke, C.M., Shaw, G., Lester, J.N., Collins, C.D., 2004. Determination of solid-liquid
443 partition coefficients (K_d) for diazinon, propetamphos and *cis*- permethrin: implications
444 for sheep dip disposal. Sci. Total. Environ. 329, 197-213.
445 Day, K.E., Scott, I.M., 1990. Use of acetylcholinesterase activity to detect sublethal toxicity
446 in stream invertebrates exposed to low concentrations of organophosphate insecticides.
447 Aquat. Toxicol. 18, 101-114.
448 Diamantino, T.C., Guilhermino, L., Almeida, E., Soares, A.M.V.M., 2000. Toxicity of
449 sodium molybdate and sodium dichromate to *Daphnia magna* Straus evaluated in acute,
450 chronic and acetylcholinesterase inhibition tests. Ecotoxicol. Environ. Saf. 45, 253-259.
451 Eisler, R., 1986. Diazinon hazards to fish, wildlife, and invertebrates: a synoptic review.
452 Biological report no. 85.; U.S. Fish and Wildlife Service, Laurel, USA.
453 *http://www.pwrc.usgs.gov/infobase/eisler/CHR_9_Diazinon.pdf*
454 Elbert, A., Becker, B., Hartwig, J., Erdelen, C., 1991. Imidacloprid- a new systemic
455 insecticide. Pflanzenschutz-Nachr. 44, 113-136.
456 Ellman, L.G., Courtney, K.D., Andres, V.Jr., Featherstone, R.M., 1961. A new and rapid
457 colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-
458 95.

459 Felsot, A.S., Cone, W., Yu, J., Ruppert, J.R., 1998. Distribution of imidacloprid in soil
460 following subsurface drip chemigation. Bull. Environ. Contam. Toxicol. 60, 363-370.
461 Felsot, A.S., Ruppert, J.R, 2002. Imidacloprid residues in Wilapa Bay (Washington State)
462 water and sediment following application for control of burrowing shrimp. J. Agric. Food
463 Chem. 50, 4417- 4423.
464 Fernandez-Casalderrey, A., Ferrando, M.D., Andreu-Moliner, E., 1995. Chronic toxicity of
465 diazinon to *Daphnia magna*: Effects on survival, reproduction and growth. Toxicol.
466 Environ. Chem. 49, 25-32.
467 Ferrando, M.D., Alarcon, V., Fernandez- Casalderrey, A., Gamon, M., Andreu- Moliner, E.,
468 1992. Persistence of some pesticides in the aquatic environment. Bull. Environ. Contam.
469 Toxicol. 48, 7474-755.
470 Fossen, M., 2006. Environmental fate of imidacloprid. California department of pesticide
471 regulation. *http://www.cdpr.ca.gov/docs/empm/pubs/fatememo/Imidclprdfate2.pdf*
472 Gälli, R., Rich, H.W., Scholtz, R., 1994. Toxicity of organophosphate insecticides and their
473 metabolites to the water flea *Daphnia magna,* the Microtox test and an
474 acetylcholinesterase inhibition test. Aquat. Toxicol. 30, 259-269.
475 Gallindo-Reyes, J.G., Venezia, D.L., Lazcano-Alvarez, G., Rivas-Mendoza, H., 2000.
476 Enzymatic and osmoregulative alterations in white shrimp *Litopenaeus vannamei* exposed
477 to pesticides. Chemosphere 40, 233-237.
478 Gonzalez-Pradas, E., Fernandez- Perez, M., Villafranca-Sanchez, M., Flores-Cespedes, F.,
479 1999. Mobility of imidacloprid from alginate-bentonite controlled-release formulations in
480 greenhouse soils. Pestic. Sci. 55, 1109- 1115.
481 Graebing, P., Chib, J.S., 2004. Soil photolysis in a moisture- and temperature-controlled
482 environment. 2. Insecticides. J. Agric. Food Chem. 52, 2606-2614.

Guilhermino, L., Lopes, M.C., Carvalho, A.P., Soares, A.M.V.M., 1996. Acetylcholinesterase activity in juveniles of *Daphnia magna* Straus. Bull. Environ. Contam. Toxicol. 57, 979-985.

Gupta, S., Gajbhiye, V.T., Kalpana, N.P., Agnihotri, 2002. Leaching behaviour of imidacloprid formulations in soil. Bull. Environ. Contam. Toxicol. 68, 502- 508.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases, the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

Hall, L.W., 2003. Analysis of diazinon monitoring data from the Sacramento and Feather river watersheds: 1991-2001. Environ. Monit. Assess. 86, 233-253.

Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine. Oxford University Press, New York, USA.

ISO 6341:1996. Water Quality –Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea)-acute toxicity test in ISO 6341, Cor.1(98). International Organisation for Standardisation, Geneve, Switzerland.

ISO/IEC 17025:1999. General requirements for the competence of testing and calibration laboratories. International Organisation for Standardisation, Geneve, Switzerland.

ISO 10706:2000. Water quality - Determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea). International Organisation for Standardisation, Geneve, Switzerland.

Jemec, A., Drobne, D., Tišler, T., Trebše, P., Roš, M., Sepčić, K., 2007. The applicability of acetylcholinesterase and glutathione S-transferase in *Daphnia magna* toxicity test. Comp. Biochem. Physiol. 144 C, 303-309.

Kagabu, S., Medej, S., 1995. Stability comparison of imidacloprid and related compounds under simulated sunlight, hydrolysis conditions, and to oxygen. Biosci. Biotech. Biochem. 59, 980-985.

508 Keizer, J., D'Agostino, G., Nagel, R., Volpe, T., Gnemi, P., Vittozzi, L., 1995. Enzymological
509 differences of AChE and diazinon hepatic metabolism: correlation of in vitro data with the
510 selective toxicity of diazinon to fish species. Sci. Total. Environ. 171, 213-220.
511 Knowles, C.O., McKee, M.J., 1987. Protein and nucleic acid content in *Daphnia magna*
512 during chronic exposure to cadmium. Ecotoxicol. Environ. Saf. 13, 290-300.
513 Konstantinou, I.K., Hela, D.G., Albanis, T.A., 2006. The status of pesticide pollution in
514 surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels.
515 Environ. Pollut. 141, 555-570.
516 Krohn, J., Hellpointner, E., 2002. Environmental fate of imidacloprid. Pflanzenschutz-Nachr.
517 55, 1-25.
518 Kühn, R., Pattard, M., Pernack, K.D., Winter, A., 1989. Results of the harmful effects of
519 water pollutants to *Daphnia magna* in the 21 d reproduction test. Wat. Res. 23, 501-510.
520 Lee, S.K., Freitag, D., Steinberg, C., Kettrup, A., Kim, Y.H., 1993. Effects of dissolved humic
521 materials on acute toxicity of some organic chemicals to aquatic organisms. Wat. Res. 27,
522 199-204.
523 Matsuda, K., Buckingham, S.D., Kleier, D., Rauh, J.J., Grauso, M., Sattelle, D.B., 2001.
524 Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends
525 Pharmacol. Sci. 22, 573- 580.
526 Moza, P.N., Hustert, K., Feicht, E., Kettrup, A., 1998. Photolysis of imidacloprid in aqueous
527 solution. Chemosphere 36, 497-502.
528 Nemeth-Konda, L., Füleky, Gy., Morovjan, Gy., Csokan, P., 2002. Sorption behaviour of
529 acetochlor, atrazine, carbendazim, diazinon, imidacloprid and isoproturon on Hungarian
530 agricultural soil. Chemosphere 48, 545-552.
531 Oi, M., 1999. Time-dependent sorption of imidacloprid in two different soils. J. Agric. Food
532 Chem. 47, 327-332.

533 PAN Pesticides database, 2006. *http:// www.pesticideinfo.org*

534 Pfeuffer, R.J., Matson, F., 2001. Pesticide surface water quality report: March 2001 sampling
535 event. South Florida Water Management District, USA.
536 *http://www.sfwmd.gov/curre/pest/P9911rpt.pdf*

537 Printes, L.B., Callaghan, A., 2003. Interclonal variability in *Daphnia* acetylcholinesterase
538 activity: The implications for its applicability as a biomarker. Environ. Toxicol. Chem. 22,
539 2042-2047.

540 Sanchez, M., Ferrando, M.D., Sancho, E., Andreu-Moliner, E., 1998. Evaluation of a *Daphnia*
541 *magna* renewal life-cycle test method with diazinon. J. Environ. Sci. Health, Part B,
542 Pestic. Food Contam. Agric. Wastes 33, 785-797.

543 Sarkar, M.A., Biswas, P.K., Roy, S., Kole, R.K., Chowdhury, A., 1999. Effect of pH and type
544 of formulation on the persistence of imidacloprid in water. Bull. Environ. Contam.
545 Toxicol. 63, 604-609.

546 SERA (Syracuse Environmental Research Associates, Inc.), 2005. Imidacloprid-Human
547 health and ecological risk assessment-final report; prepared for USDA, Forest Service,
548 USA (SERA TR 05-43-24-03a).
549 *http://www.fs.fed.us/foresthealth/pesticide/risk_assessments/122805_Imidacloprid.pdf*

550 Song, M.Y., Stark, J.D., Brown, J.J., 1997. Comparative toxicity of four insecticides,
551 including imidacloprid and tebufenozide, to four aquatic arthropods. Environ. Toxicol.
552 Chem. 16, 2494-2500.

553 Stanek, K., Drobne, D., Trebše, P., 2006. Linkage of biomarkers along levels of biological
554 complexity in juvenile and adult diazinon fed terrestrial isopod (*Porcellio scaber*,
555 Isopoda, Crustacea). Chemosphere 64, 1745-1752.

556 TDC Environmental, 2003. Insecticide market trends and potential water quality implications.
557 *http://www.swrcb.ca.gov/rwqcb2/TMDL/urbcrksdiazinon/Final_Report.pdf*

558 Tomizawa, M., Casida, J.E., 2003. Selective toxicity of neonicotinoids attributable to
559 specificity of insect and mammalian nicotinic receptors. Annu. Rev. Entomol. 48, 339-
560 364.
561 Tomizawa, M., Casida, J.E., 2005. Neonicotinoid insecticide toxicology: Mechanisms of
562 selective action. Annu. Rev. Pharmacol. Toxicol. 45, 247-268.
563 Tomlin, C.D.S., 1997. The pesticide manual. The British Crop Protection Council, UK.
564 U.S. EPA, 1999. Office of prevention, pesticides and toxic substances. EFED RED chapter
565 for diazinon, Case # 818962. *http://www.epa.gov/pesticides/op/diazinon/efedrisk.pdf*
566 U.S. EPA, 2004. Office of prevention, pesticides and toxic substances. EPA 738-R-04-006,
567 Diazinon, interim reregistration eligibility decision.
568 *http://www.epa.gov/oppsrrd1/REDs/diazinon_ired.pdf*
569 U.S. Geological Survey, 2003. Lake Wales Ridge ground water monitoring study.
570 *http://fisc.er.usgs.gov/Lake_Wales_Ridge/index.html*
571 Wamhoff, H., Schneider, V., 1999. Photodegradation of imidacloprid. J. Agric. Food Chem.
572 47, 1730-1734.
573 Watanabe, H., Grismer, M.E., 2001. Diazinon transport through inter-row vegetative filter
574 strips: micro-ecosystem modeling. J. Hydrol. 247, 183-199.
575 WHO, 2005. The WHO recommended classification of pesticides by hazard and guidelines to
576 classification: 2004. *http://www.inchem.org/documents/pds/pdsother/class.pdf*
577 Young, B., Blakemore, G., 1990. 21-days chronic static renewal toxicity of NTN 33893 to
578 *Daphnia magna*: Lab project No. 38346:100247. Unpublished study prepared by
579 Analytical Bio-Chemistry Labs., Inc., MRID 42055321.
580 Zheng, W., Liu, W., 1999. Kinetics and mechanism of the hydrolysis of imidacloprid. Pestic.
581 Sci. 55, 482-485.
582

FIGURE CAPTIONS

Fig. 1. Effects of IMI and Confidor SL 200 on the reproduction (number of neonates per female) and survival of *D. magna.* Data for reproduction are shown as mean of six replicates ± standard error of mean, and for survival as mean of three replicates. Data were fitted using sigmoid curves with the following slopes: -2.36 ± 0.42 and 1.81 ± 1.52 for the reproduction and survival of IMI, respectively, and -2.88 ± 1.00 and 2.63 ± 1.36 for the reproduction and survival of Confidor SL 200, respectively (95% confidence interval). The values for control unexposed animals were not included when fitting the data, but they are shown on graphs for comparison.

Fig.2. GST, CAT, ChE activities and protein content in *D. magna* exposed to IMI (2a), Confidor SL 200 (2b), and diazinon (2c) (mean of six replicates ± standard error of mean). Data for daphnids exposed to IMI and Confidor SL 200 were fitted using sigmoid curves. The values for control unexposed animals were not included when fitting the data, but they are shown on graphs for comparison. The slopes of the sigmoid curves for the GST, CAT, ChE activities and protein content in daphnids exposed to IMI were: -1.39 ± 1.39, -1.32 ± 1.56, -2.24 ± 2.28 and -2.44 ± 1.22, respectively, and in the case of Confidor SL 200: -0.98 ± 3.31, -1.12 ± 3.69, - 1.68 ± 3.656 and -2.00 ± 3.25, respectively (95% confidence interval). Enzyme units (EU) were defined as: the amount of ChE that hydrolyses 0.01 nmoles of acetylthiocholine min^{-1}, the amount of CAT that degrades 1 μmole of hydrogen peroxide min^{-1}, and the amount of GST that conjugates 1 nmole of reduced glutathione min^{-1}.

Fig.1

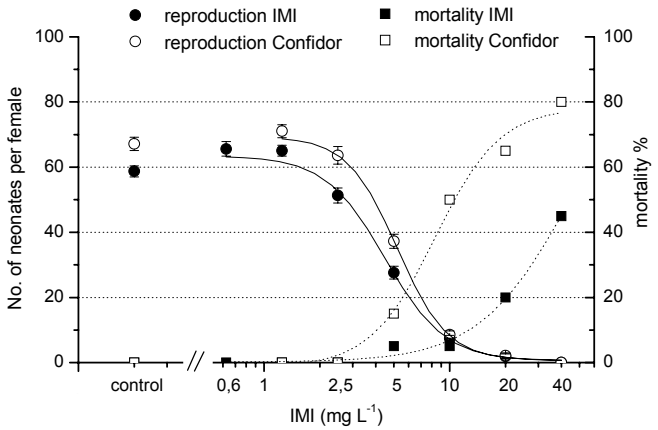
reproduction IMI
reproduction Confidor
mortality IMI
mortality Confidor
No. of neonates per female
mortality %
control
0,6
1
2,5
5
10
20
40
IMI (mg L-1)

Fig.2a

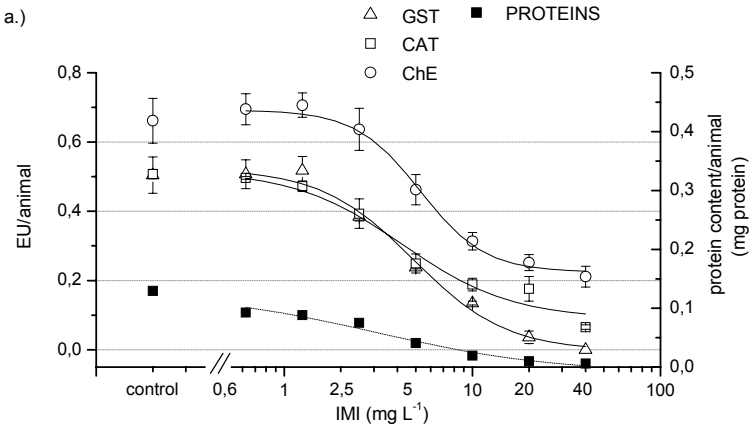
a.)
GST
CAT
ChE
PROTEINS
EU/animal
protein content/animal (mg protein)
control
0,6
1
2,5
5
10
20
40
100
IMI (mg L-1)

Fig.2b

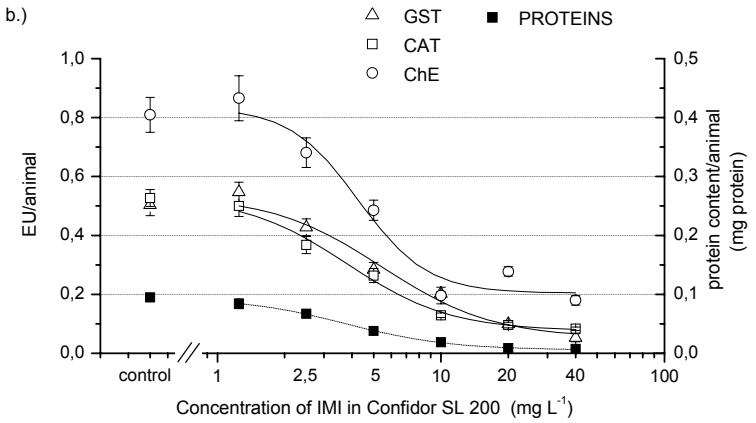

Fig.2c

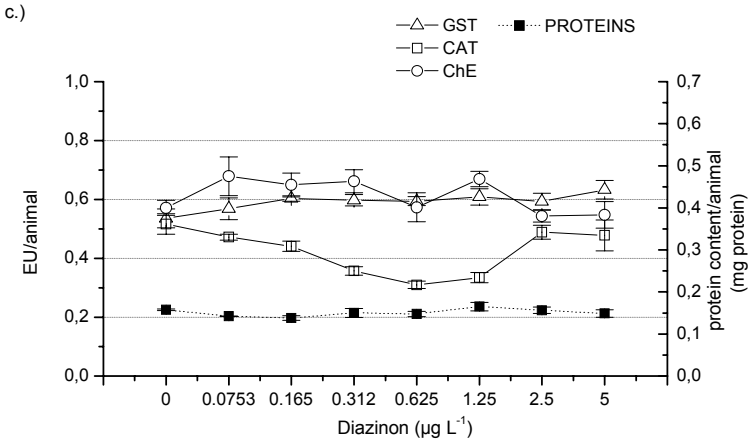

Table 1. Properties of IMI and diazinon

	DIAZINON	Ref. №	**IMI**	Ref. №
First introduced commercially	1952; J.R. Geigy S.A. (Novartis Crop Protection AG)	28	1991; Bayer AG and Nihon Tokushu Noyaku Seizo KK	28
PHYSICO-CHEMICAL PROPERTIES:				
Water solubility at 20 ºC (mg L^{-1})	60	28	a.) 610 b.) 514	a.) 28 b.) 11
K_{oc} (soil organic carbon-water partitioning coefficient)	a.) 1589 (20 °C) b.) 1520 c.) 851±180	a.) 19 b.) 26 c.) 6	a.) 210 (20 °C) b.) 249-268 c.) 109-411 (20 °C)	a.) 19 b.) 20 c.) 16
Log K_{ow} (octanol-water partition coefficient)	a.) 3.14 (20 °C) b.) 3.3 (25 °C) c.) 3.81 (20 °C)	a.) 28 b.) 26 c.) 19	a.) 0.57 (22 °C) b.) 0.92 (20 °C) c.) 0.589 (22 °C)	a.) 28 b.) 19 c.) 14
Average application rate (kg of active ingredient ha^{-1})	a.) 3.0-3.1 (orchard) b.) 0.5 (foliar); 4 (soil); 1-3 (fruit)	a.) 5 b.) 30	a.) 0.3-0.5 (soil)	a.) 24
ENVIRONMENTAL FATE:				
Detected aquatic concentrations (µg L^{-1})	a.) 3.34 (Salinas river, California, USA) b.) 6.8 (Sacramento river watershed, USA) c.) 0.775 (Greece rivers, EU) d.) 24.6 (Vicinity of pesticide factory, Egypt)	a.) 3 b.) 13 c.) 15 d.) 1	a.) 1.6 (sea Wilapa Bay, USA) b.) 1 (surface water, Florida, USA) c.) 14 (Lake Wales Ridge, USA) d.) 6.7 (ground water, New York, USA)	a.) 8 b.) 22 c.) 31 d.) 11
Estimated aquatic concentrations	8.89-429 µg L^{-1} (depends on the type of application on the crop)	30	a.) 36.04 µg L^{-1} (acute surface water exposure); 17.24 µg L^{-1} (chronic surface water exposure) b.) 22 µg L^{-1} (accidental direct spray in a pond or stream); 1.8-7.3 mg L^{-1} (accidental spill in a small pond)	a.) 11 b.) 24
Aqueous photolysis DT_{50}	140 d	26	a.) 3 h (simulated sunlight, 30 °C) b.) 1.2 h (d H_2O, λ = 290 nm, 24 °C) c.) 0.7 h (d H_2O, λ = 280 nm); 2.1 h (Confidor; d H_2O, λ = 280 nm) d.) 1 h (d H_2O, simulated sunlight)	a.) 14 b.) 18 c.) 32 d.) 16
Hydrolysis DT_{50} (d)	a.) 12 (pH5.0); 138 (pH 7.0); 77 (pH 7.7) b.) 3 (natural water pH 9.0, 12 h photoperiod) c.) 5, highly depends on pH	a.) 30 b.) 10 c.) 26	a.) 168 (26 °C, pH 4.7, 7.7, 9.0) b.) 90 (20 °C, pH 3, 5, 7) c.) > 30 d.) 37.5 (Confidor 200 SL); 41 (Gaucho 70 WS) (pH 7.0, 30 °C)	a.) 14 b.) 36 c.) 11 d.) 23
Soil photolysis DT_{50} (d)	5	26	a.) 39 b.) 38.9	a.) 16 b.) 11
Soil anaerobic DT_{50} (d)	17	26	27.1	11
Soil aerobic DT_{50} (d)	39	26	a.) 156 b.) 997	a.) 16 b.) 11
Field dissipation DT_{50} (d)	a.) 5.4-27 (lower value in moist, irradiated sandy soil) b.) 7-87.5 (lower value in non-sterile sandy loam) c.) 5-20 d.) 3-13	a.) 12 b.) 33 c.) 30 d.) 26	a.) 190 (no vegetation), 45 (vegetation); 180 (sandy and silt loam) b.) 74-156 (20 °C, bare soil); 30 -160 (sediments) c.) 27-229	a.) 20 b.) 16 c.) 11
TOXICITY:				
WHO classification	II = moderately hazardous	34	II = moderately hazardous	34
<u>Fish:</u> 1.) Rainbow trout *Oncorhynchus mykiss*	1a.) LC_{50} (96 h) = 90-400 µg L^{-1} 1b.) LC_{50} (96 h) = 20 µg L^{-1} 1c.) LC_{50} (96 h) = 90-400 µg L^{-1}	1a.) 29 1b.) 26 1c.) 7	1.a) LC_{50} (96 h) = 211 mg L^{-1} 1.b) LC_{50} (96 h) > 83 mg L^{-1} -LC_{50} (96 h) = 211 mg L^{-1} -LOLC (96 h) = 64 mg L^{-1}	1a.) 11 1b.) 24

			-LOLC (96 h) = 281 mg L^{-1}	
2.) Bluegill *Lepomis machrochirus*	2a.) LC_{50} (96 h) = 136 µg L^{-1}; 168 µg L^{-1}; 460 µg L^{-1} 2b.) LC_{50} (96 h) = 120-670 µg L^{-1}	2a) 29 2b.) 7	2.) LC_{50} (96 h) >105 mg L^{-1} -LOLC (96 h) = 42 mg L^{-1}	2.) 24
3.) Zebrafish *Danio rerio*	3.) LC_{50} (96 h) = 10 mg L^{-1}	3.) 17	3) LC_{50} (96 h) = 241 mg L^{-1}	3.) 21
Aquatic invertebrates: 1.) Water flea *Daphnia magna*	1a.) LC_{50} (48 h) = 0.96 µg L^{-1} 1b.)LC_{50} (48 h) = 0.83; 1.1 µg L^{-1} 1c.) EC_{50} (48 h) = 0.9 µg L^{-1} 1d.)NOEC $_{repr.}$ (21 d) = 5 µg L^{-1} 1f.) LOEC $_{repr.}$ (21 d) = 0.15- 0.25 µg L^{-1}	1a) 28 1b.) 29 1c.) 17 1d.) 27 1f.) 9	1a.) LC_{50} (48 h) = 85 mg L^{-1} 1b.) LC_{50} (48 h) = 10.4 mg L^{-1} 1c.) EC_{50} (48 h) = 56.6 mg L^{-1} 1d.) LOEC $_{repr.}$ (21 d) = 7.3 mg L^{-1} 1f.) LOEC $_{repr.}$ (21 d) = mg L^{-1}	1a.) 11 1b.) 25 1c.) 21 1d) 35 1f.) 27
2.) Amphipod *Hyalella azteca*	2a.) LC_{50} (96 h) = 6.51 µg L^{-1} 2b.) LC_{50} (96 h) = 4.3 µg L^{-1}	2a.) 4 2b.) 2	2.) LC_{50} (96 h) (juveniles) = 0.526 mg L^{-1} -LC_{50} (96 h) (14-21 d old) = 51.8 mg L^{-1} -LC_{50} (96 h) (7-21 d old) = 94.8 mg L^{-1} -LOEC $_{immobility}$ (96 h) (juveniles) = 0.00097 mg L^{-1} -LOLC (96 h) (14-21 d old) = 43.8 mg L^{-1} - NOEC $_{immobility}$ (96 h) (7-21 d old) = 94.8 mg L^{-1}	2.) 24
3.) Midge *Chironomus tentans*	3.) LC_{50} (96 h) = 10.7 µg L^{-1}	3.) 4	3.) LC_{50} (96 h) (2^{nd} instar) = 0.0105 mg L^{-1}; - LOLC (96 h)(2^{nd} instar) = 0.00339 mg L^{-1}	3.) 24

Abbreviations: DT_{50} (half life); EC_{50} (median effective concentration for immobility); LC_{50} (median lethal concentration); LOEC $_{repr. / immobility}$ (lowest observed effect concentration for reproduction/immobility); LOLC (lowest observed lethal concentration); NOEC $_{repr./ immobility}$ (no observed effect concentration for reproduction/immobility); OC (organic carbon).

References: Abdel-Halim et al., 2006 (1); Anderson and Lydy, 2002 (2), Anderson et al., 2003 (3); Ankley and Collyard, 1995 (4); Cobb et al., 2000 (5); Cooke et al. 2004 (6); Eisler, 1986 (7); Felsot and Ruppert, 2002 (8); Fernandez-Casalderrey et al., 1995 (9); Ferrando et al., 1992 (10); Fossen, 2006 (11); Graebing and Chib, 2004 (12); Hall, 2003 (13); Kagabu and Medej, 1995 (14); Konstantinou et al., 2006 (15); Krohn and Hellpointner, 2002 (16); Lee et al., 1993 (17); Moza et al., 1998 (18); Nemeth-Konda et al., 2002 (19); Oi, 1999 (20); Our laboratory, unpublished (21); Pfeuffer and Matson, 2001 (22); Sarkar et al., 1999 (23); SERA, 2005 (24); Song et al., 1997 (25); TDC Environmental, 2003 (26); This study (27); Tomlin, 1997 (28); U.S. EPA, 1999 (29); U.S. EPA, 2004 (30); U.S. Geological Survey, 2003 (31); Wamhoff and Schneider, 1999 (32); Watanabe and Grismer, 2001 (33); WHO, 2005 (34); Young and Blakemore, 1990 (35); Zheng and Liu, 1999 (36).

Table 2. 21 d LOEC (lowest observed effect concentration) values for biochemical and reproduction data, and 21 d LOLC (lowest observed lethal concentration) values for mortality data. The following concentrations of analytical grade IMI: 0, 0.625, 1.25, 2.5, 5, 10, 20, 40 mg L^{-1}; IMI formulated as Confidor SL 200: 0, 1.25, 2.5, 5, 10, 20, 40 mg L^{-1} of IMI, and diazinon: 0, 0.0753, 0.165, 0.312, 0.625, 1.25, 2.5, 5, 8 µg L^{-1} were tested.

Endpoint / Chemical	№ of neonates per adult	Brood size	Days to first brood	№ of broods per adult	Mortality	GST	CAT	ChE	Total protein content
IMI (mg L^{-1})	2.5	5	5	10	40	2.5	5	10	1.25
Confidor SL 200 (in mg L^{-1} of IMI)	5	5	5	10	10	5	2.5	5	2.5
Diazinon (µg L^{-1})	5<(L)OEC< 8	5<(L)OEC< 8	5<(L)OEC< 8	5<(L)OEC< 8	8[1]	5<(L)OEC< 8	n.d.[2]	5<(L)OEC< 8	5<(L)OEC< 8

[1] 100% mortality of adult daphnids was observed at this concentration. At lower tested concentration (5 µg/L), no statistically significant mortality was detected (≤20%).

[2] LOEC could not be determined due to insignificant trend

Table 3. Calculated risk quotients (RQ) of tested chemicals for *D. magna* (based on 21 d LOEC (lowest observed effect concentration) for biochemical parameters (bio. param.), reproduction, and 21 d LOLC (lowest observed lethal concentration) for survival).

Chemical	Exposure concentration (µg L^{-1}) [2]	$RQ_{bio.\ param.}$	$RQ_{reproduction}$	$RQ_{survival}$
IMI	a.) highest detected: 14	a.) 0.0112	a.) 0.0056	a.) 0.00035
	b.) lowest detected: 1	b.) 0.0008	b.) 0.0004	b.) 0.000025
	c.) estimated (chronic surface water): 17.24	c.) 0.0138	c.) 0.0069	c.) 0.00043
	d.) estimated (accidental spill): 7300	d.) 5.8[1]	d.) 3[1]	d.) 0.183
IMI in Confidor SL 200	a.) highest detected: 14	a.) 0.0056	a.) 0.0028	a.) 0.0014
	b.) lowest detected: 1	b.) 0.0004	b.) 0.0002	b.) 0.0001
	c.) estimated (chronic surface water): 17.24	c.) 0.0069	c.) 0.00345	c.) 0.00172
	d.) estimated (accidental spill): 7300	d.) 3[1]	d.) 1.46[1]	d.) 0.73
Diazinon	a.) highest detected: 24.6	a.) > 3.1[1]	a.) > 3.1[1]	a.) 3.1[1]
	b.) lowest detected: 0.775	b.) > 0.097	b.) > 0.097	b.) 0.097
	c.) estimated: 429	c.) > 53.6[1]	c.) > 53.6[1]	c.) 53.6[1]

[1] Potentially chronically hazardous to *D. magna* (RQ>1) (U.S. EPA; 2004)

[2] Please refer to Table 1 for references on exposure concentrations

Table 4. Calculated risk quotients (RQ) of diazinon and IMI for freshwater invertebrates and vertebrates (fish) (based on LC_{50} (96 h))

Chemical	Exposure concentration (µg L^{-1})[2]	RQ: Aquatic invertebrates[2]	RQ: Vertebrates (fish)[2]
IMI	a.) highest detected: 14	0.0266-1.4[1]	0.000058-0.000168
	b.) lowest detected: 1	0.0019-0.095	0.0000041-0.000012
	d.) estimated (accidental spill): 7300	13.8[1]-695.2[1]	0.031-0.0879
Diazinon	a.) highest detected: 24.6	0.145-117[1]	0.0025-1.23[1]
	b.) lowest detected: 0.775	0.0046-3.7[1]	0.000077-0.0387
	c.) estimated: 429	2.52[1]-2043[1]	0.0429-21.45[1]

[1] Potentially acutely hazardous to selected aquatic organisms (RQ>0.5) (U.S. EPA; 2004)

[2] Please refer to Table 1 and TDC Environmental (2003) for references on exposure concentrations and LC_{50} (96 h) data, respectively.