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**Pesticide residues in grapes from integrated production
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Pesticide residues in grapes from vineyards included in
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Pesticide residues in grapes from integrated production of the Slovene origin

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Pesticide residues in grapes from vineyards included in integrated pest management in Slovenia

Abstract

Although the list of pesticides used in integrated pest management (IPM) in grape growing and their annual application rates are limited, we are still confronted with the problem of pesticide residues in grapes. This paper presents the results of pesticide monitoring of 47 samples of wine grapes (*Vitis vinifera* L.) from the 2006 vintage from the vineyards included in IPM. The grape samples were analysed for the presence of 67 pesticides. Among them, 20 were allowed in IPM in 2006. Grapes were sampled at harvest. Two internal analytical methods were used for determination of pesticides: GC-MS method for determination of dithiocarbamates and a multi-residue GC-MS method. One grape sample (2.1%) contained no residues or they were below limit of detection, 28 samples (59.6%) contained residues lower or equal than maximum residue levels (MRLs) and 18 samples (38.3%) exceeded national MRLs for cyprodinil (concentration range was 0.03-0.40 mg kg⁻¹ of cyprodinil) and fludioxonil (concentration was 0.03 mgkg⁻¹ of fludioxonil). Multiple residues were found in 41 samples (87.2%). The highest number of pesticides detected per sample was seven. No violation of pesticides allowed in IPM was observed. Folpet (97.9%), cyprodinil (51.1%), dithiocarbamates (44.7%), chlorothalonil (23.4%), chlorpyrifos (19.1%) and pyrimethanil (14.9%) were the most frequently found pesticides in grapes. The risk assessment showed that the exceeded concentrations of cyprodinil and fludioxonil did not represent any risk for consumer's health (National Estimate of Short Term Intake in % of Acceptable Daily Intake was below 100%).

Keywords: pesticides, plant protection products, GC-MS, grapevine

Introduction

Numerous analytical methods for determining pesticide residues in grapes have been published until now. GC and HPLC are the two most powerful techniques. GC was cited in the literature on many occasions. Oliva et al. (1999) developed an on-line micro-extraction for determination of chlorpyrifos, penconazole, fenarimol, vinclozolin and metalaxyl in grapes. For determination, electron capture detection and mass selective detection were used (Oliva et al. 1999). The method was extended to some other organophosphorus insecticides (Oliva et al. 2000). Navarro et al. (2000) used on-line micro-extraction technique, followed by nitrogen-phosphorus or electron-capture detection and confirmation by mass spectrometric detection to determine fungicide residues in grapes. An automated matrix solid-phase dispersion method (MSPD) with gas chromatography-mass spectrometry detection for eleven pesticides in grapes was developed by Maria Kristenson et al. (2001). Sandra et al. (2003) described stir bar sorptive extraction technique followed by gas chromatography-mass spectrometry for determination of pesticides in grapes. Recently, methods using classical liquid-liquid extraction were published. Fernández González et al. (2003) described organic solvent extraction with dichloromethane-acetone followed by gas chromatography with mass spectrometric detection used for determination of fungicide residues in grapes. Angioni et al. (2005) described extraction with hexane and analysis of zoxamide with mass spectrometry. Sharif et al. (2006) described extraction of grape samples with ethyl acetate, clean-up with three different sorbents and final determination by gas chromatography and electron-capture detection.

HPLC covers polar and thermally labile compounds which cannot be determined by GC. Miliadis et al. (1999) described a method for extraction with ethyl acetate-sodium sulphate,

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3 clean-up by solid-phase extraction (SPE) on silica sorbent and determination with a UV diode
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5 array detector for benzoylurea insecticides in grapes. Taylor et al. (2002), Mol et al. (2003)
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7 and Jansson et al. (2004) described methods for determination of pesticide residues in grapes
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9 after extraction with ethyl acetate by liquid chromatography tandem mass spectrometry. Otero
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11 et al. (2003) described method for determination of 14 fungicides in grapes with liquid-liquid
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13 extraction with dichloromethane-acetone mixture, clean-up with SPE and determination by
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15 liquid chromatography and diode array detection. HPLC with diode array detection was also
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17 used by Tiexeria (2004), after extraction with methanol and SPE clean-up. Juan-García et al.
18
19 (2004) developed a LC/MS method based on SPE and stir-bar sorptive extraction (SBSE).
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21 Finally, Pang et al. (2006) described extraction with acetonitrile, 3-cartridge SPE and
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23 determination separately by GC/MS and LC/MS in grapes.
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32 We used a multi-residue method for the determination of 66 pesticide residues, which
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34 included extraction with mixture of acetone, petroleum ether and dichloromethane, clean-up
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36 on gel permeation chromatography and GC/MS detection. The other method was used for
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38 determination of dithiocarbamates. In this method samples are heated in a two-phase system
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40 isooctane/stannous (II) chloride in diluted hydrochloric acid. The produced carbon disulfide is
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42 dissolved in the organic phase (isooctane) and determined by GC/MS.
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48 Pesticide residues found in wine grape samples from IPM were determined at harvest, in
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50 accordance with the Law on Plant Protection Products (Official Gazette of the Republic of
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52 Slovenia no. 98/04) and Regulation on Residues of Plant Protection Products Found in and on
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54 Agricultural Commodities and Products (Official Gazette of the Republic of Slovenia, no.
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56 84/04).
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3 In Slovenia one half (8554 ha) of registered vineyards were included in IPM. The main
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5 restrictions in that production system rely on grapevine fertilization, plant protection and
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7 vineyard soil management. In IPM the use of some environmentally dangerous pesticides are
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9 not allowed and only those from the lists found in Technical Guidelines for IPM in Grape
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11 Growing, published by the Government, can be used. The main goal of IPM is a proper use of
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13 pesticides regarding the concentrations at application, the maximum number of applications,
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15 the consideration to pre-harvest interval and the certification of sprayers. Consideration of
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17 prognostic forecasts and economic losses in the case of plant infection is a very important step
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19 in the decision making process for the application of pesticides. In IPM the quantity of copper
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21 applied must not exceed $5 \text{ kg ha}^{-1} \text{ year}^{-1}$ and the use of some derivatives of iso-cyclic compounds
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23 (tolylfluanid, dinocap), dithiocarbamates (mancozeb, metiram, propineb), inhibitors of the
24
25 ergosterol biosynthesis (IBE fungicides: myclobutanil, penconazole, tebuconazole),
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27 fungicides against grey mould (boscalid, fenhexamide, iprodione, fludioxonil, cyprodinil,
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29 pyrimethanil), insecticides, folpet and chlorothalonil is limited (Technical Guidelines for IPM
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31 in Grape Growing in 2006, 2006). The main benefits of IPM grape growing are lower
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33 incidence and lower levels of residues in grapes and consequently also in wines.
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43 The results of monitoring are used for: determination of harmonisation with the legally
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45 prescribed MRLs, determination of harmonisation of IPM with good agricultural practice,
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47 determination of origin or cause of residues determined and risk assessment for samples
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49 which exceeded MRLs.
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54 The incidence and concentrations of pesticide residues in harvested grapes depends on the
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56 pests and diseases of grapevine typical for each vine growing region, type of grape production
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58 (conventional, IPM, organic), pesticide concentrations at application, time period and climatic
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3 conditions from the last spraying until harvest. There are only a few papers dealing with a
4 wide range of pesticide residues in wine grapes. On the other hand, more data exist for
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conditions from the last spraying until harvest. There are only a few papers dealing with a wide range of pesticide residues in wine grapes. On the other hand, more data exist for dissipation rates of some pesticides in wine grapes (Navarro et al. 2001; Cabras and Angioni, 2000) and from grapes to wine (Cabras et al. 1999; Navarro et al. 1999). It is also interesting that there is a lack of results concerning pesticide residues in grapes and wines for different types of grape growing systems, either for IPM or for organic viticulture. These results are obviously needed to support consumer confidence in the growing market of wines from sustainable viticulture. A fifteen years old review of Farris et al. (1992) describes the results of pesticide residues in grapes. Most of the pesticides listed in the paper are still registered but some of them were forbidden for use in IPM in Slovenia (i.e. triadimefon, dimethoate, carbaryl, deltamethrin, thiabendazole). The results of the studies mentioned above are more closely examined and compared with our results in the Results and Discussion section of this paper. Many authors also showed that a proper use of pesticides does not cause an exceedance of MRLs in grapes (Cabras et al. 1999; Farris et al. 1992; Navarro et al. 1999). However, Soleas and Goldberg (2000) assayed 26 pesticides in 1827 raw grape juices prior to fermentation, in which grapes were the source of the contamination. Pesticides found most frequently in their samples were folpet, captan, guthion, carbaryl and dimethoate. Only the first one is allowed for use in IPM of grape growing in Slovenia. The goal of the present paper was therefore to investigate the incidence and concentrations of pesticide residues in grapes from vineyards included in IPM and to examine the important part of safety assurance in grape growing, which is the first stage of the winemaking process.

Materials and methods

Sampling

The sampling of wine grapes was random and it was carried out in all three vine growing regions in Slovenia (Podravje, Posavje and Primorska). Grape samples were taken directly in the vineyard after the pre-harvest interval of the pesticides elapsed. Agricultural inspectors performed the sampling in autumn 2006.

Analytical methods

Multi-residue method

The following 66 pesticides were analysed using multiresidual method: acephate, aldrin, azinphos-methyl, azoxystrobin, bifenthrin, bromopropylate, bupirimate, captan, carbaryl, carbofuran, chlorothalonil, chlorpropham, chlorpyrifos, chlorpyrifos-methyl, cyhalotrin-lambda, cypermethrin, cyprodinil, DDT, deltamethrin, diazinon, dichlofluanid, dimethoate, diphenylamine, endosulfan, endrin, fenitrothion, fenthion, fludioxonil, folpet, HCH- α , heptachlor, heptenophos, imazalil, iprodione, kresoxim-methyl, lindane, malathion, mecarbam, metalaxyl, methamidophos, methidathion, myclobutanil, omethoate, oxydemeton-methyl, parathion, penconazole, permethrin, phorate, phosalone, pirimicarb, pirimiphos-methyl, procymidone, propargite, propyzamide, pyridaphenthion, pyrimethanil, quinalphos, spiroxamine, thiabendazole, tolclofos-methyl, tolylfluanid, triadimefon, triadimenol, triazophos, trifloxystrobin and vinclozolin.

The extraction procedure and determination by GC/MS of the multi-residue method is described elsewhere (Baša Česnik and Gregorčič, 2003; Fillion et al., 2000; Makovi and

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3 McMahon, 1999; Thier and Zeumer, 1987; Thier and Zeumer, 1992;). The multi-residue
4 method was validated on the strawberry matrix. This method was accredited by the French
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6 method was validated on the strawberry matrix. This method was accredited by the French
7
8 accreditation body COFRAC and covers the validation and accreditation for the whole group
9

10 Method used for determination of dithiocarbamates

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12 The extraction procedure and determination by GC/MS and validation of this method is
13 described elsewhere (Baša Česnik and Gregorčič, 2006).
14

15 *Quality assurance*

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17 Each series of analyses included two spiked samples of commodity analysed. For batch
18 acceptance recoveries had to be 70 – 110 %. The recoveries were allowed to be higher for
19 those pesticides which were not found in the samples. The accuracy of methods has been
20 verified since 2002 by participating in the French interlaboratory proficiency scheme BIPEA
21 (Bureau Interprofessionnel d'Etudes Analytiques). Z-scores for eight grape juice samples for
22 44 measurements from 2003 to 2004 were mainly below ± 2 . Unsatisfactory results were
23 obtained on five occasions; the highest absolute z-score was 2.54. Taking into account the
24 measurement uncertainty the “false” results fell within the tolerance limits. We have also
25 participated in the CRL European Proficiency Test since 2004. In 2005 our work was verified
26 and accredited by French accreditation body (COFRAC) for a multi-residue method on leafy
27 vegetables, root and tuber vegetables and flour. In 2006 the scope of accreditation was
28 expanded to pome fruit and berries and small fruit. In 2006 we also accredited a method for
29 the determination of dithiocarbamates for the groups: pome fruit, berries and small fruit, leafy
30 vegetables, root and tuber vegetables, fruiting vegetables and cereals.
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Results and discussion

Validation of the multi-residue method for 66 pesticides on berries and small fruit matrix showed that limits of detection ranged from 0.0004–0.02 mg kg⁻¹, limits of quantification range from 0.001-0.08 mg kg⁻¹, repeatability standard deviations ranged from 5-10% and recoveries at spiking levels from 0.013-0.2 mg kg⁻¹ ranged from 93.4-102.5%. Validation of method for determination of dithiocarbamates on berries and small fruit matrix showed that the limit of detection was 0.02 mg kg⁻¹, limit of quantification was 0.05 mg kg⁻¹, repeatability standard deviation was 0.5%, and the recovery at spiking level 2 mg kg⁻¹ was 99.6%.

Out of the total of 47 grape samples analysed, 18 samples (38.3%) exceeded the maximum residue limits (cyprodinil and fludioxonil), 28 samples (59.6%) contained residues lower than maximum residue limits, and in one grape sample (2.1%) residues were not found. Forty-one (87.2%) out of the total of 47 grape samples analysed contained residues of two or more pesticides. Residues of two pesticides were determined in 14 samples (29.8%) of which MRL was exceeded by 4 samples (8.5%) with the values from 0.12 – 0.29 mg kg⁻¹ (cyprodinil), residues of three pesticides were determined in 13 samples (27.7%) of which MRL was exceeded by 4 samples (8.5%) with the values from 0.25 - 0.40 mg kg⁻¹ (cyprodinil), residues of 4 pesticides were determined in 7 samples (14.9%), of which MRL was exceeded by 7 samples (14.9%) with the values from 0.07 – 0.34 mg kg⁻¹ (cyprodinil), residues of 5 pesticides were determined in 5 samples (10.6%) of which MRL was exceeded by one sample (2.1%) at a level of 0.24 mg kg⁻¹ (cyprodinil), residues of 6 pesticides were determined in one sample (2.1%) which exceeded MRL at a level of 0.14 mg kg⁻¹ (cyprodinil), residues of 7 pesticides were determined in one sample (2.1%) which exceeded MRL at levels of 0.10 mg kg⁻¹ (cyprodinil) and 0.03 mg kg⁻¹ (fludioxonil).

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6 Pesticides, their concentration levels determined in grape samples and MRLs are presented in
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8 table 1.
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10 [Insert table 1 about here]
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15 Pesticides sought during the analyses and allowed in IPM were: azoxystrobin, chlorothalonil,
16 chlorpyrifos, chlorpyrifos-methyl, cyprodinil, dithiocarbamates, endosulfan, fludioxonil,
17 folpet, iprodione, kresoxim-methyl, metalaxyl, myclobutanil, penconazole, phosalone,
18 pyrimethanil, spiromamine, tolylfluanid, triadimenol and trifloxystrobin. In the grape samples
19 we found 14 of the pesticides listed. No violation of pesticides allowed in IPM was observed;
20 we could conclude that viticulturists used only those allowed in the Technical Guidelines for
21 IPM in 2006.
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34 It was expected that among pesticides found the most frequently in grapes would be folpet
35 (97.9% of analysed samples), cyprodinil (51.1%), dithiocarbamates (44.7%), chlorothalonil
36 (23.4%), chlorpyrifos (19.1%) and pyrimethanil (14.9%). Namely, folpet, dithiocarbamates
37 and chlorothalonil belong to the group of contact fungicides against downy mildew
38 (*Plasmopara viticola* (Berk et Curtis ex. De bary)) Berl. et de Toni) and are frequently used
39 for two or three last applications in a growing season. Cyprodinil and pyrimethanil are
40 anilinopyrimidines used against grey mould (*Botryotinia fuckeliana* de Bary (Whetzel)).
41 During growing season, grapes are usually sprayed two or three times with those fungicides
42 and the last application coincides with the beginning of grape ripening (growth stage 35-36
43 according to Eichhorn-Lorenz scale). Chlorpyrifos is an organophosphorus insecticide used
44 against grapevine moths (*Eupoecilia ambiguella* Hübn. and *Lobesia botrana* Den. & Schiff.)
45 and is also applied at the beginning of grape ripening (Technical Guidelines for IPM in Grape
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3 Growing in 2006, 2006). All pesticides mentioned above, except cyprodinil, were determined
4 within EC or national MRLs (pyrimethanil). Viticulturists accurately considered the
5 guidelines for the use of pesticides, especially their application rates and pre-harvest intervals.
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7 Concentrations of the most frequently found pesticides in wine grapes (Table 1) are
8 comparable to those already reported by Farris et al. (1992) (folpet (0.50 mg kg⁻¹),
9 dithiocarbamates (0.90-1.60 mg kg⁻¹), chlorothalonil (0.23-0.60 mg kg⁻¹), Navarro et al.
10 (2001) (chlorpyrifos (0.14 mg kg⁻¹), Cabras et al. (1997) (cyprodinil (1.03 mg kg⁻¹),
11 pyrimethanil (1.11 mg kg⁻¹)) and Rial Otero et al. (2003) ((folpet (0.10-0.60 mg kg⁻¹),
12 cyprodinil (0.14-1.45 mg kg⁻¹), pyrimethanil (2.33 mg kg⁻¹)), where all the values were below
13 EC or national MRLs. A comparison of our results with the results of Soleas and Goldberg
14 (2000) that assayed 26 pesticides in 1827 raw juices prior to fermentation from 9 major wine-
15 producing countries shows that there is some important difference in the list of detected
16 pesticides. Among the same pesticides assayed in the grape samples we did not detect some of
17 the very problematic insecticides i.e. endosulfan (allowed in IPM), dimethoate, malathion,
18 parathion and carbaryl.

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41 The fungicides that exceeded the national MRL in Slovenia are cyprodinil in 18 samples
42 (38.3%) and fludioxonil in one sample (2.1%). The national MRL for both compounds is 0.02
43 mg kg⁻¹. The highest content of cyprodinil was 0.40 mg kg⁻¹ and of fludioxonil 0.03 mg kg⁻¹.
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48 The European Community MRLs for both substances have not been determined yet. The
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Codex Alimentarius MRLs are 3 mg kg⁻¹ for cyprodinil and 2 mg kg⁻¹ for fludioxonil. The risk
assessment performed with Pesticide Safety Directorate (PSD, York, UK), model for acute
exposure for cyprodinil at concentration level 0.40 mg kg⁻¹ and Acceptable Daily Intake
(ADI) 0.03 mgkg⁻¹ body weight⁻¹ day⁻¹ (Acute Reference Dose for cyprodinil was not
determined) showed that National Estimate of Short Term Intake (NESTI) expressed in ADI

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3 percentage ranged from 2.5% for 7-10 years old children to 31.6% for adults. The risk
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5 assessment performed with PSD model for acute exposure for fludixonil at concentration
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7 level 0.03 mg kg⁻¹ and ADI 0.40 mg kg⁻¹ body weight⁻¹ day⁻¹ (Acute Reference Dose for
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9 fludixonil was not determined) showed that NESTI expressed in ADI percentage ranged from
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11 0.0% for 1-10 years old children and residential elderly people to 0.2% for adults and
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13 vegetarians. ADIs were found on the internet
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15 (http://europa.eu.int/comm/food/plant/protection/evaluation/index_en.htm), Status of active
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17 substances under EU review (doc. 3010)) as well as the PSD model for acute exposure
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19 (<http://www.pesticides.gov.uk/approvals.asp?id=1687>). The risk assessment showed that the
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21 exceeded grape samples did not present any risk for health (NESTI in % of ADI was below
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23 100%) and are therefore safe for the consumers. Perhaps the national MRLs should be
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25 reconsidered. Additionally, all the grape samples were wine grapes which were further
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27 processed into wine. The published results (Cabras et al., 1999; Navarro et al., 1999) and also
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29 our unpublished results showed that pesticide concentrations during the vinification process
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31 are importantly reduced. For example, in spite of the fact that the cyprodinil concentration in
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33 grapes of another experiment was above (0.25 mg kg⁻¹) the national MRL, it was not found in
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35 the processed wine and therefore its starting concentration presented no risk for the wine
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37 consumers.
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48 **Conclusions**

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50 Although the list of pesticides used in IPM in grape growing and their annual application rates
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52 are limited, we are still confronted with the problem of pesticide residues in grapes. This
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54 paper presents the results of pesticide monitoring of 47 samples of wine grapes (*Vitis vinifera*
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56 L.) from vineyards included in IPM for the presence of 67 pesticides. In IPM the use of some
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58 environmentally and consumer dangerous pesticides is not allowed and only those from the
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3 lists in Technical Guidelines for IPM in Grape Growing can be used. The main goal of IPM is
4 therefore a proper use of pesticides regarding the prescribed concentrations, the maximum
5 number of treatments and the consideration of the pre-harvest interval. Our results for the
6 2006 vintage showed that aims mentioned above were fully achieved as far as the list of
7 permitted pesticides is concerned. The same applies to the residue concentrations where
8 neither EC nor national MRLs were exceeded except for cyprodinil and fludioxonil. The
9 residues in samples were exceeded due to the very low national MRLs. The risk assessment
10 showed that grape samples that exceeded MRLs do not present any risk for consumer's health
11 (NESTI in ADI percentage was below 100%). It has to be stressed that wine grapes are
12 subjected to the vinification process in which pesticide residues are further reduced or
13 eliminated. The cases of fungicides exceeding the national MRLs should be probably further
14 examined and discussed.

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34 We could conclude that the use of permitted pesticides, the consideration of pesticide
35 concentrations and the pre-harvest interval importantly diminish the health risk for consumers
36 and provide safety assurance in IPM wine grape growing. Therefore, such a type of wine
37 grape production does not represent any cause of concern in Slovenia.

38 39 40 41 42 43 44 45 **Acknowledgements**

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Table 1: Pesticides found in grape samples

Pesticide	No. of samples	Proportion (%)	Range (mgkg ⁻¹)	MRL (mgkg ⁻¹)
Azoxystrobin	4	8.5	0.02-0.04	2
Chlorothalonil	11	23.4	0.01-0.73	3
Chlorpyrifos	9	19.1	0.02-0.13	0.5
Cyprodinil	24	51.1	0.01-0.40	0.02*
Dithiocarbamates	21	44.7	0.06-1.63	2
Fludioxonil	2	4.3	0.02-0.03	0.02*
Folpet	46	97.9	0.02-6.0	10
Iprodione	2	4.3	0.01-0.30	10
Kresoxim-methyl	1	2.1	0.01	1
Metalaxyl	5	10.6	0.05-0.18	1
Myclobutanil	1	2.1	0.02	1
Phosalone	2	4.3	0.01-0.02	1
Pyrimethanil	7	14.9	0.01-0.53	3*
Trifloxystrobin	3	6.4	0.02-0.09	5

*national MRLs of the Republic of Slovenia, all others are EC MRLs