POTENTIAL DERMAL EXPOSURE OF FLORISTS TO FUNGICIDE RESIDUES ON FLOWERS AND RISK ASSESSMENT

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SUMMARY

Flowers are susceptible to many pests and diseases. Therefore, they can be sprayed several times during their growth considering that no MRL are set for flowers. High levels of pesticide residues potentially expose daily the florists who handle cut flowers and possibly could endanger their health. A study was carried out to evaluate the risk for florists exposed to fungicide residues during normal professional tasks. Cotton gloves were distributed to 20 florists (two pairs to each florist) and worn during two consecutive half days during normal professional tasks (from min 2 hours to max 3 hours/day) to measure their potential dermal exposure (PDE). Samples were analyzed with a multi-residue (QuEChERS) method validated by a laboratory accredited for pesticide residues and with a combination of gas and liquid chromatography tandem mass spectrometry. It appears from the results that a total of 54 fungicides with different toxicity classes were detected on cotton gloves. An average of 15.53 mg/kg fungicide residues per glove sample was measured. Six of 54 are suspected of causing cancer after prolonged or repeated exposure. Boscalid was both the active substance for which the highest maximum and average concentrations (26.21 and 3.47 mg/kg, respectively). Famoxadone had the most critical PDE (156% AOEL for the maximum concentration). As a consequence, this study leads to conclude that Belgian florists, who worked for several years and handled a large number of flowers contaminated by high concentrations of pesticide residues, are exposed daily with a potential effect on their health. This suggests that safety standards should be set for residue levels on cut flowers.

Key words: dermal exposure, risk assessment, cut flowers, florists.

INTRODUCTION

As in any intensive culture, flowers require the use of a wide range of fungicides to control fungal diseases, which can damage production and marketability and to stay competitive in both national and international markets (Cooper Dobson, 2007). Fungicides use provides many benefits to ornamental producers, including the consistent availability, the reliable control. They are in general less expensive than alternatives and may reduce plant pathogenic transmission (Bethke and Cloyd, 2009). However, a large majority of growers still consider pesticides, as vital tool in floriculture that can improve and maintain crop more productive and profitable in the face of costs and quantities of floral products, meaning they will be able to market large quantities of floral products with an acceptable quality and relatively modest prices. The vast majority of European florists is not actively engaged in social and environmental standards, not as a purchasing criterion, nor in their communication towards consumers (Rikken, 2010). Moreover, the flower industry practices have been completely unregulated with lack of maximum residue limits (MRL) for flowers explains that, unlike other crops which are harvested for consumption, resulting in the use of highly toxic chemicals, regularly up to harvesting. A recent study, conducted on cut flowers (roses, gerberas, and chrysanthemums) sold in Belgium, showed that flowers are heavily contaminated by fungicide residues. The most frequently detected active substance was the fungicide fluopyram (42 samples out of 90) (Toumi *et al.*, 2016a). In another study, the fungicide dodemorph was the most frequently detected active substance with the highest maximum concentration (41.9 mg/kg) measured in the rose samples (Toumi *et al.*, 2016b). The great majority of fungicides sprayed on cut flowers are actually dislodgeable by human contact (hands, gloves, and clothing). These active substances are persistent, fat-soluble and can be absorbed through skin contact. Consequently, Belgian florists who handle a large number of flowers, daily and for several hours can potentially be exposed to residual deposits of pesticides and possibly endanger their health (Toumi *et al.*, 2016a). Pesticides have been closely linked to a wide range of serious health concerns for exposed floriculturist operators and workers, ranging from short-term impacts such as weakness and fatigue, muscle pain, chills and fever, blurred vision, dizziness and headache (Lu, 2005) to chronic impacts like cancer (Fleming *et al.*, 1999), genetic damage (Munnia *et al.*, 1999; Bolognesi, 2003) and reproductive harm (abortion, prematurity, and congenital malformations) (Restrepo *et al.*, 1990). Following the exposure problems, dermal risk assessment is deemed necessary for Belgian florists handling daily contaminated flowers and preparing bouquets. Assessing the amount of pesticide residues on workers' hands is often the main measure of dermal exposure. Hand exposure often accounts for a significant proportion of the total exposure has been documented many times (US EPA 1986; OECD 1997). As a matter of fact, gloves, hand washes or hand wipes (McCurdy *et al.*, 1994; Baldi *et al.*, 2006; Aprea *et al.*, 2009; Baldi et al., 2014) are often used to sample dermal hand exposure. Gloves worn during normal professional tasks act as a reservoir for active substances that come into contact with the skin (Brouwer *et al.*, 1992 a, b and c; Jurewicz *et al.*, 2009; Li *et al.*, 2011). In order to assess the risk for professionals exposed to pesticide residues on flowers, a study has been carried out with a group of florist on a voluntary basis.

MATERIALS AND METHODS

To evaluate the potential dermal exposure of florists preparing bouquets, twenty samples of cotton gloves (two pairs of cotton gloves/sample) were distributed to Belgian volunteer florists and worn during two consecutive half days when handling flowers (from min 2 h to max 3 h/day) to measure the potential transfer of pesticides from treated flowers to hands. The two pairs were collected as a single sample (four gloves/sample), weighed, cut in small pieces with scissors and stored in freezing bags at −18 °C. The samples were kept for no more than three days before being taken to the analytical laboratory (transport by road from Gembloux to Ghent).

The residual fungicide deposits on the gloves were analysed in a laboratory holding a BELAC accreditation to ISO/CEI 17025 for pesticide residues (PRIMORIS, Technologiepark 2/3, B-9052 Zwijnaarde – Ghent). PRIMORIS is a private, accredited and officially recognised service laboratory. Samples were analyzed using a multi-residue Quick Easy Cheap Effective Rugged Safe (QuEChERS) method validated by the laboratory for the analysis of residues in foodstuffs. The extraction procedure is based on the AOAC (Association of Official Analytical Chemists) Official Method 2007.01 (Lehotay, 2007). According to the active substances to be determined, the pesticide residue concentration was quantified by GC-MS/MS or LC-MS/MS (gas chromatography or liquid chromatography tandem mass spectrometry). Gas chromatography was used to analyse relatively small, thermally stable, volatile, non-polar molecules. Liquid chromatography was used to analyse larger, thermolabile, non-volatile, polar molecules. The combination of both methods allows the analysis of approximately 500 active substances in a single analysis. For most of the active substances, the quantification limit was ≤ 0.01 mg/kg. Therefore, the analytical results were corrected accordingly for all active substances with a recovery ratio between 50–130% (only few substances had a percentage of recovery below or above these values; in this case, the results remain uncorrected in the tables).

The potential dermal exposure (PDE) values were estimated as the amount of pesticide residues with low adhesion that were transferred from flowers to gloves. For each active substance, PDE was calculated as follows:

PDE (in mg a.s./kg bw per day) = $((C(_{T\ (h)})\ (mg/kg) \times GW\ (kg)) \times 3)/bw\ (kg)$

where C is the concentration of active substance in the sub-sample, GW is the average weight of the cotton gloves samples (57 g $\pm$ 0.17 g), T is the task duration during the trial (2 h), and bw is the body weight (60 kg). A total task duration value of 6 h/day was used to assess the dermal exposure of florists. A recent publication mentioned that 60% of the Belgian florists worked between 6 and 7 h/day (Toumi *et al.*, 2016a). A default body weight (bw) value of 60 kg is used in line with the recent EFSA Guidance Document to cover a range of professionally exposed adults (EFSA, 2014). The risk characterisation is obtained as the ratio of the exposure level to the reference value of each active substance, the AOEL (Acceptable Operator Exposure Level; in mg a.s./kg·bw per day). Several prediction levels of the PDE were considered, including the mean, 75th percentile, 90th percentile, and the maximum (worst case) (in mg/kg bw per day) to assess the risk for florists.

RESULTS AND DISCUSSION

FUNGICIDE RESIDUES CONCENTRATIONS ON COTTON GLOVES

All glove samples appeared to be contaminated by high levels of fungicide residues for most active substances. A total of 54 fungicides were identified, with an average of about 21.05 a.s./sample and an average total fungicide residue concentrations per glove sample of 15.53 mg/kg (Table 1).

Seventeen active substances (azoxystrobine, benomyl, boscalid, cyprodinil, dimethomorph, dodemorph, famoxadone, fenhexamid, fludioxonil, fluopyram, iprodione, mandipropamid, methoxyfenozide, prochloraz, procymidone, propamocarb and spiroxamine) are the most frequently detected fungicides. They are present on more than 12 of the 20 samples (60%).

Table 1. Total number of a.s. detected per sample and fungicide residues concentrations in 20 samples of gloves

Samples from florists	Total number of detected a.s. (LOQ ≤ 0.01 mg/kg)	Total fungicide residues concentrations (mg/kg)

Sample N°1	25	89.87
Sample N°2	24	19.21
Sample N°3	27	2.69
Sample N°4	31	27.46
Sample N°5	36	35.50
Sample N°6	23	7.41
Sample N°7	13	4.71
Sample N°8	34	33.92
Sample N°9	17	6.11
Sample N°10	9	1.08
Sample N°11	25	18.41
Sample N°12	15	6.18
Sample N°13	33	20.89
Sample N°14	10	3.31
Sample N°15	25	16.89
Sample N°16	18	4.56
Sample N°17	14	0.96
Sample N°18	21	6.10
Sample N°19	13	4.10
Sample N°20	8	1.18
Mean	**21.05**	**15.53**
Median	**22.00**	**6.15**

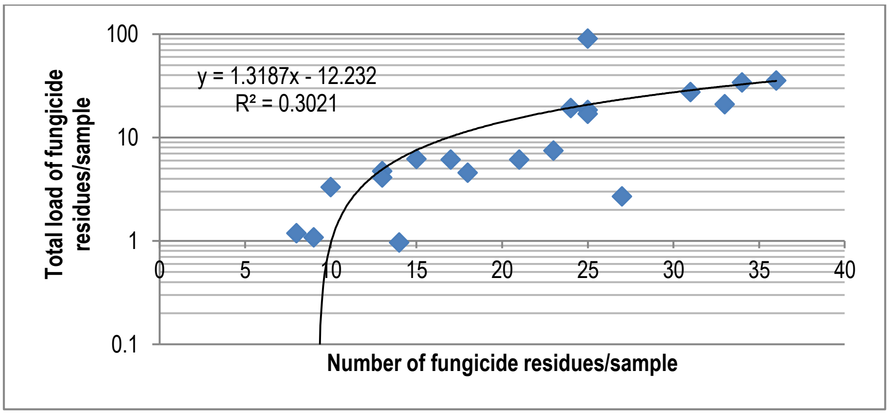

Figure 1. Variation in the total load of pesticides (mg/kg)/sample according to the number of active substances detected/sample.

RISK ASSESSMENT FOR FLORISTS

The risk is generated by combination of the "hazard" (mode of action; acute and chronic toxicity of a.s.) and "exposure" (concentration levels on flowers; routes of exposure: oral or dermal). The main route of exposure to plant protection products is the oral route, yet most exposures to operators, workers, bystanders and residents will be via dermal and / or inhalation routes. Therefore, it is necessary to assess the exposure for the operators (i.e. applicators, crop-workers, harvesters) for the different likely routes of exposure.

HAZARD IDENTIFICATION AND CHARACTERISATION

Classification of active substances according to the average and maximum concentrations

Five active substances (boscalid, iprodione, mandipropamid,fludioxonil and fenhexamid) of the 54 detected fungicides in the 20 cotton gloves samples have an average concentration higher than 1 mg/kg. Boscalid was the active substance with both the highest maximum and average concentrations (26.21 and 3.47 mg/kg, respectively).

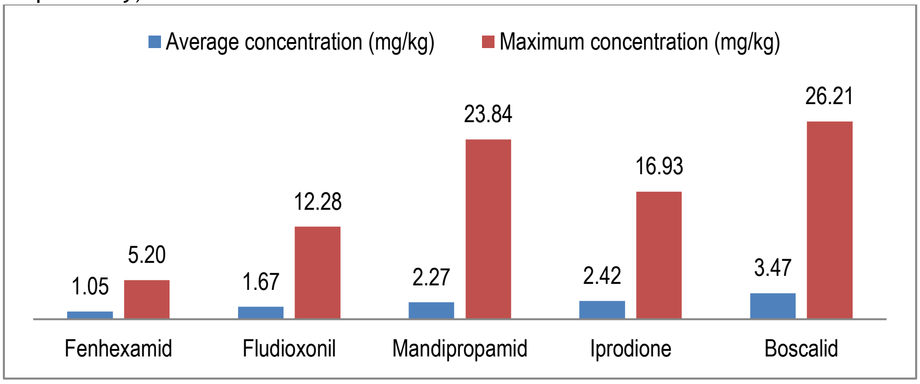

Figure 2. Fungicides with the highest average and maximum residues concentrations measured on 20 samples of cotton gloves.

Classification of active substances according to the chemical family

Of the 54 detected active substances, most of the fungicides belong to the following chemical groups: triazoles (12 a.s); strobilurins (6 a.s); anilinopyrimidines and benzimidazoles (3 a.s. each); carbamates, dicarboximides and phenylpyrroles (2 a.s. each). Pesticides from those groups are known for their toxicological properties (action on the nervous system after exposure; acute toxicity, etc).

Classification of active substances according to the EU Pesticides Database

As the florists handle the flowers every day in the course of their work, they are exposed to plant protection products like other "operators". Worker exposure rates can be similar to those of operators. However, it should be taken into account that workers are often exposed for extended periods of time and usually don't take any protective measures. The "Acceptable Operator Exposure Level" (AOEL) is the reference value to consider for professionals exposed to pesticides. AOEL is defined in Regulation (EC) 1107/2009 as "... *the maximum amount of active substance to which the operator may be exposed without any adverse health effects*". AOEL values relate to the internal (absorbed) dose available for systemic distribution from any route of absorption and are expressed as internal levels (mg/kg bw/day). When the operator exposure remains below this limit, the risk for them is considered as "acceptable" (Regulation (EC) 1107/2009) (Table 2).

The active substances can also be classified on their hazard category according to the CLP regulation (for "Classification, Labelling and Packaging") (Regulation (EC) 1272/2008) is a European Union regulation from 2008, which aligns the European Union system of classification, labelling and packaging of chemical substances and mixtures to the Globally Harmonised System (GHS). It is expected to facilitate global trade and the harmonised communication of hazard information of chemicals and to promote regulatory efficiency (Table 3).

Table 2 . Number of fungicides detected on the gloves worn by florists classified according to their AOEL values *(Source: EU Pesticides Database 2017, European Commission/DG HEALTH and Regulation (EC) 1107/2009)*

AOEL values (mg/kg bw/d)	Number of active substances
[0.001-0.01 [	4
[0.01-0.1 [	27
[0.1-1[	20
>1	1
No AOEL*	2

**Active substances which have no AOEL values; not assessed at European level.*

Table 3. Number of fungicides detected on the gloves worn by florists classified by hazard category according to the CLP regulation *(Source: Regulation (EC) 1272/2008)*

Class	Category	Code (Hazard)	Number of a.s. in the category
Acute toxicity	Category 2	H330: Fatal if inhaled	1
	Category 3	H331: Toxic if inhaled	3
	Category 4	H302: Harmful if swallowed	12
		H312: Harmful in contact with skin	1
		H332: Harmful if inhaled	4
Carcinogenicity	Category 2	H351:Suspected of causing cancer	6
Serious eye damage/ eye irritation	Category 1	H318: Causes serious eye damage	4
	Category 2	H319: Causes serious eye irritation	1
Germ cell mutagenicity	Category 1,1A or 1B	H340: May cause genetic defects	1
	Category 2	H341: Suspected of causing genetic defects	1
Reproductive toxicity	Category 1,1A or 1B	H360: May damage fertility or the unborn child.	2
	Category 2	H361: Suspected of damaging fertility or the unborn child.	6

Sensitisation of the respiratory tract or the skin	Skin sensitisers category 1,1A or 1B	H317: May cause an allergic skin reaction	14
Skin corrosion / irritation	Category 1,1A or 1B	H314: Causes severe skin burns and eye damage	1
	Category 2	H315: Causes skin irritation	4
Specific target organ toxicity (single exposure)	Category 3	H335: May cause respiratory irritation	2
Specific target organ toxicity (repeated exposure)	Category 2	H373: May cause damage to organs through prolonged or repeated exposure	2

According to the CLP classification (Table 3), the majority of detected active substances have potential hazardous chronic effects. Twenty one have an acute toxicity. Six are also suspected of causing cancer. Many fungicides detected in the glove samples may affect the skin of the florists after exposure by contact (allergic reaction: 14; skin irritation: 4; harmful in contact with skin: 1; severe skin burns and eye damage: 1). Furthermore, five cause serious eye damage and eye irritation. Moreover, one active substance may cause genetic defects and one is suspected of a similar effect. In addition, eight active substances have a reproductive toxicity, two may cause respiratory irritation and two may cause damage to organs through prolonged or repeated exposure.

DERMAL EXPOSURE ASSESSMENT AND RISK CHARACTERISATION

Risk characterization is the fourth step of the risk assessment process, integrating information from the hazard characterization and the exposure assessment to produce scientific advice for risk managers (Renwick *et al.*, 2003). Risk is estimated by comparing potential dermal exposure to the Acceptable Operator Exposure Level (AOEL).

Table 4. Classification of the active substances (and metabolites) according to the PDE expressed in percentages of their respective AOEL values (mean, 75th percentile, 90th percentile, and maximum) for all identified fungicides in the 20 samples of gloves worn by the Belgian florists.

PDE in % of AOEL	PDE (Mean)	PDE (75th P)	PDE(90th P)	PDE (Max)
[0-50% [	Ametoctradin Azoxystrobine Benomyl (carbendazim) Bitertanol Boscalid Bupirimate Captan (tetrahydrophtalimide) Chlorothalonil Cyproconazole Cyprodinil Difenoconazole	Ametoctradin Azoxystrobine Benomyl (carbendazim) Bitertanol Boscalid Bupirimate Captan (tetrahydrophtalimide) Chlorothalonil Cyproconazole Cyprodinil Difenoconazole	Ametoctradin Azoxystrobine Benomyl (carbendazim) Bitertanol Boscalid Bupirimate Captan (tetrahydrophtalimide) Chlorothalonil Cyproconazole Cyprodinil Difenoconazole	Ametoctradin Azoxystrobine Bitertanol Bupirimate Captan (tetrahydrophtalimide) Chlorothalonil Cyproconazole Cyprodinil Difenoconazole Dimethomorph Diphenylamine Dodemorph

	Dimethomorph Diphenylamine Dodemorph Famoxadone Fenamidone Fenhexamid Fluazinam Fludioxonil Fluopicolide Fluopyram Fluoxastrobin Flusilazole Flutolanil Flutriafol Fluxapyroxad Iprodione Iprovalicarb Kresoxim-methyl Mandipropamid Mepanipyrim Metalaxyl (metalaxyl-M) Methoxyfenozide Metrafenone Myclobutanil Penconazole Picoxystrobin Prochloraz Procymidone Propamocarb Propiconazole Pyraclostrobin Pyrimethanil Spiroxamine Tebuconazole Tetraconazole Thiabendazole Thiophanate methyl Tolclofos-methyl Triadimefon(triadimenol) Trifloxystrobin Triflumizole	Dimethomorph Diphenylamine Dodemorph Famoxadone Fenamidone Fenhexamid Fluazinam Fludioxonil Fluopicolide Fluopyram Fluoxastrobin Flusilazole Flutolanil Flutriafol Fluxapyroxad Iprodione Iprovalicarb Kresoxim-methyl Mandipropamid Mepanipyrim Metalaxyl (metalaxyl-M) Methoxyfenozide Metrafenone Myclobutanil Penconazole Picoxystrobin Prochloraz Procymidone Propamocarb Propiconazole Pyraclostrobin Pyrimethanil Spiroxamine Tebuconazole Tetraconazole Thiabendazole Thiophanate methyl Tolclofos-methyl Triadimefon(triadimenol) Trifloxystrobin Triflumizole	Dimethomorph Diphenylamine Dodemorph Fenamidone Fenhexamid Fluazinam Fludioxonil Fluopicolide Fluopyram Fluoxastrobin Flusilazole Flutolanil Flutriafol Fluxapyroxad Iprodione Iprovalicarb Kresoxim-methyl Mandipropamid Mepanipyrim Metalaxyl (metalaxyl-M) Methoxyfenozide Metrafenone Myclobutanil Penconazole Picoxystrobin Prochloraz Propamocarb Propiconazole Pyraclostrobin Pyrimethanil Spiroxamine Tebuconazole Tetraconazole Thiabendazole Thiophanate methyl Tolclofos-methyl Triadimefon(triadimenol) Trifloxystrobin Triflumizole	Fenamidone Fenhexamid Fluazinam Fludioxonil Fluopicolide Fluopyram Fluoxastrobin Flusilazole Flutolanil Flutriafol Fluxapyroxad Iprodione Iprovalicarb Kresoxim-methyl Mandipropamid Mepanipyrim Metalaxyl (metalaxyl-M) Methoxyfenozide Metrafenone Myclobutanil Penconazole Picoxystrobin Prochloraz Propamocarb Propiconazole Pyraclostrobin Pyrimethanil Spiroxamine Tebuconazole Tetraconazole Thiabendazole Thiophanate methyl Tolclofos-methyl Triadimefon(triadimenol) Trifloxystrobin Triflumizole
[50-100% [	-	-	Procymidone Famoxadone	Boscalid
≥ 100%	-	-	-	Procymidone Benomyl (carbendazim) Famoxadone

The potential dermal exposures of florists were estimated for the average, for different percentiles, and for the maximum concentration of residues in samples (Table 4). The results from the different percentiles used to estimate PDE vary by orders of magnitude. As was shown in Table 4, no fungicide exceeds the AOEL for PDE mean, PDE P75 and PDE P90 values. However, at the maximum (PDE_{MAX} or worst case) values of PDE, three fungicides (benomyl (carbendazim) (128% AOEL), famoxadone (156 % AOEL) and procymidone (100 % AOEL)) exceed the AOEL indicating risk situations.

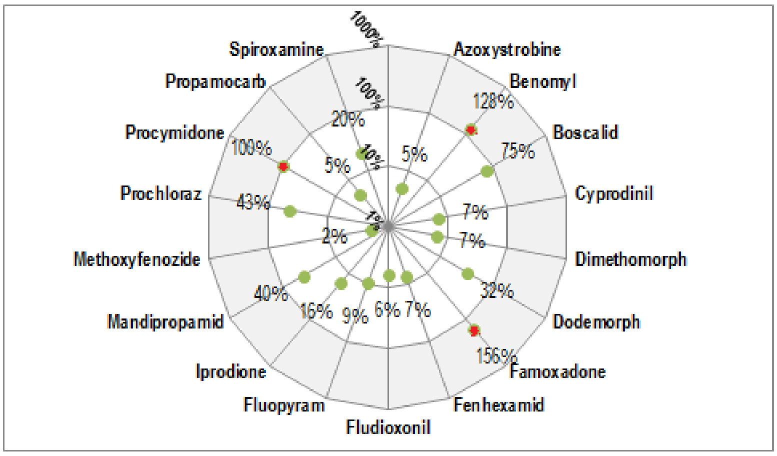

Figure 3. The maximum potential exposure (PDE_{MAX}) of the seventeen most frequently detected active substances on gloves worn by florists as a percentage of the AOEL

Three of 17 fungicides mostly detected on gloves exceed the AOEL indicating risk situations. Famoxadone has the most critical PDE (156% AOEL for the maximum concentration).

CONCLUSION

The first conclusion after analysis of the fungicide residual deposits on cotton gloves worn by florists is the high level of contamination of all samples: 54 fungicides detected, i.e. an average of almost 21 active substances/sample and a total average pesticide load of 15.53 mg/kg per cotton gloves sample. This reflects the intensive use of fungicides on cut flowers in general. As the flowers are susceptible to fungal diseases, they are regularly treated till the harvest time without any restriction on the fungicide use because there are no maximum residue limits (MRLs) for flowers, unlike other cultures. The high levels of fungicide residues in gloves worn by the florists are linked to high rates of pesticides but also to repeated sprayings during the growing season. The results from this study have shown that boscalid was the active substance for which the highest maximum and average concentrations (26.21 and 3.47 mg/kg, respectively) measured on the glove samples analysed. This study illustrates that the majority of these fungicides have an acute toxicity (21 s.a.) and potential hazardous chronic effects (carcinogenicity (6 s.a.), serious eye damage or irritation (5 s.a.), germ cell mutagenicity (2 s.a), reproductive toxicity (8 s.a.), sensitisation of the skin (14 s.a.), skin corrosion or irritation (5 s.a.), specific target organtoxicity (single exposure) (2 s.a.) and specific target organtoxicity (repeated exposure) (2 s.a.)). Concerning the PDE, no fungicide exceeds the AOEL for PDEmean, PDE P75 and PDE P90 values. However, at the maximum (or worst case) values of PDE, three fungicides (benomyl (carbendazim) (128% AOEL), famoxadone (156 % AOEL) and procymidone (100 % AOEL)) exceed the AOEL indicating risk situations. These fungicides are known for their toxicological properties and have potential hazardous chronic effects, e.g. benomyl (and its metabolite carbendazim), which is not approved in EU, may affect the skin of the florists after exposure by contact (allergic reaction, skin irritation), may cause genetic defects and respiratory irritation and may damage fertility or the unborn child. In addition, famoxadone may cause damage to organs through prolonged or repeated exposure. Thus, Belgian florists who handle a large number of flowers are at risk for exposure to pesticide residues with potential effects on their health. Moreover, it should be taken into account that the majority of florists do not wear gloves, or any other PPE, even if they spend 2 to 6 h per day handling cut flowers and preparing bouquets (Toumi *et al.*, 2016a). To better assess the risk, bio-monitoring of the florists with analysis of their blood, urines, and hairs is still to be investigated in the lab. To reduce the exposure of florists to pesticide residues, solutions could be recommended: protective gloves during handling, hygiene rules, better pesticides management at the field (IPM or even organic flower production, a potential niche market) and a quality control of imported cut flowers. Finally, it could be interesting to set up a Maximum Residue Limit for flowers to decrease the risk for professionals and all other people in contact with flowers.

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