# Efficiency of an artificial wetland and a forest buffer for pesticide pollution mitigation in a tile-drained agricultural watershed

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**Doctorat ParisTech**
**T H È S E**

**pour obtenir le grade de docteur délivré par**

**L'Institut des Sciences et Industries du Vivant et de l'Environnement (AgroParisTech)**

**Spécialité :**
**Sciences de l'eau**

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*présentée et soutenue publiquement par*

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le 12 Novembre 2010

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## ABSTRACT

# Efficiency of an artificial wetland and a forest buffer for pesticide pollution mitigation in a tile-drained agricultural watershed

As part of the ArtWET LIFE environment project (06 ENV/F/000133), two buffer zones, an artificial wetland and a forest buffer, located at Bray (France) were assessed for their potential at reducing pesticide pollution coming from a 46-ha tile-drained watershed. Because of limited land availability, treating the entire volume was not possible but focusing on the most concentrated effluents appeared to be a good strategy to achieve pesticide abatement. The three-year results showed that both systems lowered down pesticide concentrations and loads by more than 40 %. However, a wide range of efficiencies was recorded. The most contrasted results were obtained for the fairly mobile herbicide isoproturon. On-site multi-tracer experiments concluded in a 66.5-h water residence time in the artificial wetland. Delay was observed in pesticide transfer probably due to adsorption, but desorption was also suspected. Wetland sediments, wetland plants, forest soil and litter were sampled on-site. On these substrates, $^{14}C$ radio-labelled pesticides were used to study epoxiconazole (fungicide) degradation under flooded conditions as well as adsorption and desorption of isoproturon, metazachlor (herbicides) and epoxiconazole. Apart from plants, adsorption coefficients ($K_{oc}$) for the three molecules were in the upper range of literature values indicating a high sorption potential of the buffer zone substrates. Epoxiconazole showed the lowest desorption properties whereas metazachlor was more easily released from most substrates. Epoxiconazole mineralization was low and occurred at a very slow rate. Degradation occurred as attested by metabolite production. Sorption-desorption seemed an important phenomenon to consider particularly for systems with low residence time in which degradation may not have time to occur. In addition, such temporarily anoxic environment does not prevent pesticide degradation from taking place but may slow it down by a factor depending on pesticide properties.



**Keywords:** artificial wetlands, forest buffer, pesticides, pollution, mitigation.

## RESUME

# Efficacité d'une zone humide artificielle et d'une zone tampon forestière pour dissiper la pollution par les pesticides dans un bassin versant agricole drainé.

Dans le cadre du projet européen LIFE ArtWET (06 ENV/F/000133), deux zones tampons (ZTs), une zone humide artificielle (ZHA) et une zone tampon forestière (ZTF), situées à Bray (France), ont été évaluées pour leur efficacité à réduire la pollution par les pesticides venant d'un bassin versant agricole drainé de 46 ha. Traiter l'ensemble des volumes n'étant pas possible, une bonne stratégie de traitement semble être de cibler les volumes les plus concentrés en pesticides. Les trois ans de données indiquent en moyenne une réduction d'au moins 40 % des concentrations et des charges en pesticides dans les deux ZTs, bien qu'une forte variabilité ait été notée. L'isoproturon, un herbicide mobile, a donné les résultats les plus contrastés. Des expérimentations de traçage ont permis d'estimer le temps de rétention hydraulique à 66.5 h dans la ZHA. Les retards observés sur le transfert des pesticides à travers des ZTs semblent dus à l'adsorption, bien que des phénomènes de désorption soient aussi suspectés. Des sédiments et des plantes de la ZHA ainsi que du sol et de la litière de la ZTF ont été prélevés. Sur ces substrats, des molécules marquées au $^{14}C$ ont permis de suivre la dégradation de l'époxiconazole (fongicide) en systèmes eau/substrats ainsi que l'adsorption et la désorption de l'isoproturon, du metazachlore (herbicide) et de l'époxiconazole. A part pour les plantes, les coefficients d'adsorption ($K_{oc}$) des trois molécules sont dans les valeurs hautes des gammes de valeurs publiées indiquant un fort potentiel des substrats de ces ZTs pour la rétention des pesticides. La désorption est très faible pour l'epoxiconazole, mais assez élevée pour le metazachlore et l'isoproturon. La minéralisation de l'epoxiconazole est faible et lente mais des métabolites ont été observés, indiquant une dégradation partielle. L'adsorption-desorption semble être un phénomène important, notamment pour les ZTs où le temps de résidence est faible, laissant ainsi peu de temps pour la dégradation des molécules.



**Mots-clés:** zone humide artificielle, zone tampon forestière, pesticides, pollution, dissipation.

## ACKNOWLEDGEMENTS

This Ph.D has been carried out within the Hydrosystems and Bioprocesses (HBAN) research unit, at the Cemagref research institute, Parc de Tourvoie, BP44, 92163 Antony Cedex, France. It was supported by a LIFE Environment grant (06/ENV/F/000133, ArtWET project). In addition, a seven-month period was spent at the "Environnement et Grandes Cultures" (EGC) research unit at the INRA research institute, 78850 Thiverval-Grignon, France thanks to a FIRE financial support. I wish to thank all the persons belonging to these institutions who contributed to this work.

First, I am grateful to the jury members who accepted to evaluate this work: Pr. Marnik Vanclooster, Dr. Chantal Gascuel, Pr. Ralf Schulz, Dr. Rafael Muñoz-Carpena. In particular, I wish to thank Dr. Cyril Kao for introducing me with the Cemagref and the research world four years ago. I am very pleased he accepted to participate in this jury.

Foremost, I want to thank Dr. Julien Tournebize for his continuous support during all the phases of this project through very stimulating and enlightening discussions, field work and daily encouragements. I am grateful for all his contributions of time, ideas and insight in all circumstances that definitely contributed to the completion of this project.

I would like to express my gratefulness to Dr. Yves Coquet who provided extremely important support in crucial periods of this project. With his calm and professionalism, his supervision always resulted in rich and efficient exchanges.

I would also like to make a special relevance to Cédric Chaumont for his incomparable expertise in on-site material set-up, data collection and analysis, for very fruitful discussions and the good times we shared which definitely contributed to carry through with this dissertation.

Angeline Guenne played a key role in the very first 36 months of this Ph.D by helping, tirelessly, getting all the water sample analysis done… Congratulations for your patience and perseverance with The Machine! Certainly, Sylvain Moreau and Jean-Michel Bouyé were of great help in uncertainty estimations.

I am grateful to Bernard and Anthony Crespin who let us come and work on a part of their land and for their personal involvement in the project during wetland digging works, sample collection, vegetation maintenance. Similarly, this work would not have been possible without Franck Paineau (Chambre d'agriculture, Indre-et-Loire) and his regular visits to the site and the information he provided.

From the Cemagref Lyon, I received precious help from Jean-Joël Gril and Christelle Margoum in conducting field experiment, or through worthy discussion on buffer zone, design of experiments, analytical method developing.

My "Dank" also goes to Jens Lange, Tobias Schütz, Sonja Jankowfsky, Bernadette Prömse and Emil Blattmann (Institute für Hydrologie, Freiburg, Germany) who helped in preparing tracer experiments or even brave the Bray cold weather to conduct the experiments as part of the ArtWET project. Thanks also to Caroline Gregoire who made possible this LIFE ArtWET project.

It is a pleasure to thank all the students from the Cemagref who made this Ph.D possible and particularly Tanya Culhaoglu (my first great master student who also fought and beated The Machine), Benjamin Richard (my second great master student for his deep involvement in his forest study and nice field visits), Jessica Brest and Brice Siroux (who also successfully braved The Machine's mood swing), Cécile Meigné (for her professionalism, seriousness and calm during vegetation inventory and drive to the field…) and Carolina Hoyos-Hernandez (por su increíble capacidad de trabajo y continuo buen humor).

During my stay at the EGC INRA, Pierre Benoit and Valérie Bergheaud were of outstanding help to set up the experiments and analyze the data. I am grateful to Sabine Houot who accepted me in the Soil team and several of the Soil team members who punctually provided me precious assistance (Valérie Dumeny, Yolaine Goubard, Muriel Jolly, Jean-Noël Rampon) and master and Ph.D students (Maha, Clément, Nadler, Sohaib, Flavie, Karim, Matthieu and Florian) who either helped me at the lab or at least contributed in creating a nice environment to work in.

Miscellaneously, I would like to thank pre-Doc (Jérémy Pulou, good luck!) and post-Docs (Dr. Claire Billy, Dr. Hocine Henine and Dr. Corinne Fesneau) from the Cemagref for their support and outside work fun times, Nagwa Safey and Joëlle Sauce (for irreplaceable assistance in literature search), Valérie Dansin and Sophie Morin (for their crucial help in administrative tasks), Roger Martin (to have maintained my computer until the end), the Tapahs team (Yves Nédélec, Bernard Vincent, Gaëlle Tallec, Irina Ginzburg, Alain Guerin), Dr. François Birgand, FIP, "Le Lion d'Or" hotel restaurant, Anglès (81, Tarn), my new laptop, Vincent (for his advices and perspicacity in understanding my Ph.D prog(st)ress), Aurélie (for photo sessions of climbing week ends I missed…), Lucas (for the layout hot line), Gilles (for the desk)…

I have the chance to have a wonderful and understanding family. Thanks to Dad, Mom, Fabien, Fanny and Domi for your moral support and encouragements along the course of this Ph.D.

# OUTLINE

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# INTRODUCTORY CHAPTER: STATE-OF-THE-ART: BUFFER ZONES FOR PESTICIDE POLLUTION CONTROL

Surface- and ground-water quality destruction has partly incriminated agricultural pollutants (Fig. 1). New regulations have been implemented in order to improve water quality such as the European Water Framework Directive (2000/60/EC) and the French EcoPhyto Program (French government, 2008). The latter requires a 50% pesticide use reduction for 2018 compared to 2008.

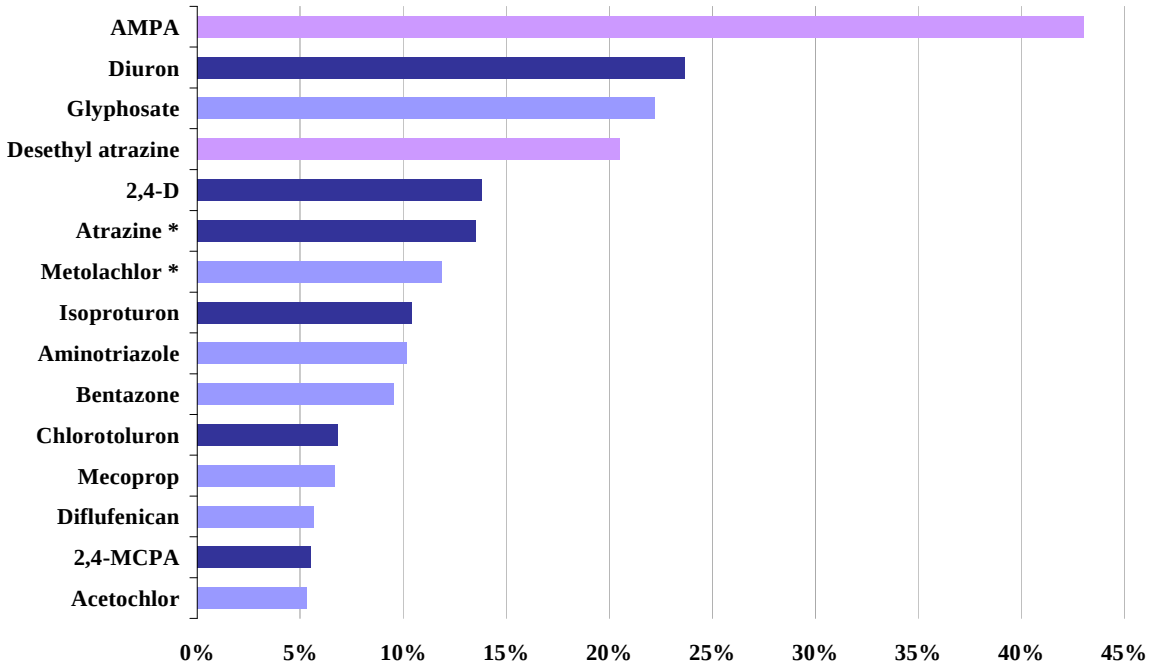



**Fig. 1: Most frequently quantified pesticide in French surface water in 2007 (IFEN, 2007b). * Molecules that were forbidden in 2007. In purple are metabolites, dark blue are substances with French Environmental Quality Standard ("Norme de Qualité Environnementale", NQE). Data come from French Agences de l'Eau.**

To comply with such regulations, a series of complementary measures should be considered and pesticide fate in the environment should be well understood. Pesticides can be mitigated through in-field and off-field measures. Implemented at the farm scale prior to or during pesticide application, the former includes active substance selection, application rate reduction, application date shifting and proper use and cleaning of pesticide spraying equipment (Reichenberger et al., 2007). As long as pesticides are used, a certain portion will transfer to natural systems. Thus, complementary measures at plot and catchment scales, such as conservation tillage on cultivated surfaces and buffer zone implementation on specific areas are needed. Surface flows, including surface runoff and drainage outflows, are accessible contrary to infiltration flows on which implementing treatment measures is difficult to perform. Buffer zones can be either existing vegetated landscape elements, or created or restored new buffer systems. This option deals with the possibility of considering the sink potential of several buffer systems to reduce pesticide pollution. The complementarities of these measures has been highlighted by van der Valk and Jolly (1992) who insisted on the fact that creating wetlands (one possible buffer system) should be part of a more comprehensive plan to reduce non point source pollution.

## 1 The ArtWET LIFE Environment project

In this context, the ArtWET LIFE Environment project (LIFE 06 ENV/F/000133), entitled “Mitigation of agricultural nonpoint source pollution and bioremediation in artificial wetland ecosystems”, started in 2006 as part of the Water Framework Directive (2000/60/EC)

implementation. The project was motivated after ascertaining that "artificial wetland ecosystems", further named "buffer zones" in the present dissertation, have ability to improve pesticide-degraded water quality but that such systems functioning is still poorly understood and lack of optimization. Coordinated by the ENGEES Strasbourg (France), the ArtWET project gathered nine partners from France, Germany and Italy, including the Cemagref (France). Demonstration sites including "artificial wetland ecosystems" like artificial wetland, forest buffer, detention ponds, vegetated ditches, outdoor bioreactor, were set up and monitored by team partners. In addition, smaller scale mesocosm systems were set up. The objective was to assess their efficiency at reducing pesticide pollution and provide guidelines to optimize their design. Results were and are still being disseminated through various media: scientific papers, international conference participation (e.g. WETPOL 2007 and 2009), meetings organized by each partner in its own language, newsletters, website (www.artwet.fr), demonstration site visits… Two guides summarizing the results of the project on sociological and technical aspects are being finalized and will be delivered by the end of 2010 to help stakeholders implement such systems.

Through the ArtWET project, this PhD program specifically focused on two on-site systems located at the outlet of an agricultural tile-drained catchment at Bray (Indre-et-Loire, France), where a three-cell in series artificial wetland and a forest buffer were monitored from late 2007 to spring 2010.

## 2 Pesticide molecule complexity

About 1000 different pesticide molecules exist and approximately 300 are in use in France encompassing an extremely wide range of complexity (INRA, 2010). Their intrinsic toxic properties can turn out to be harmful for human and ecosystems. Not only they are contaminants, but they can be pollutants as well for soil, air and surface and ground waters (Voltz et al., 2006; IFEN, 2007a). Pesticides residues can be found in vegetables and fruits that we consume. The AGRITOX or FOOTPRINT databases gather pesticide toxicity and eco-toxicity data through different parameters. For instance, they report acute toxicity values like $DL_{50}$ and $CL_{50}$, corresponding to letal dose and concentration for 50 % of a population, respectively, or chronic toxicity values like NOEC (no effect concentration) (FOOTPRINT, 2010; INRA, 2010). Pesticides are suspected to be implicated in respiratory, cancerous and nervous diseases as well as in immune and reproduction systems disorder (Saiyed et al., 2003; Ministère de la santé et des solidarités, 2004; Rekha et al., 2006). Their presence in natural environments is therefore worrying.

Pesticides are frequently characterized by their adsorption capacity and degradability. The former is assessed through an adsorption coefficient, representing the pesticide distribution between a solid and liquid phase, and being normalized to the organic carbon ($K_{oc}$, mL/g). The latter is derived from half-lives values ($DT_{50}$, d) determined at the laboratory or field scales, and corresponding to the time needed to decrease by 50 % initial pesticide concentration. However, these two parameters are not sufficient to fully explain pesticide fate in buffer zones. Other characteristics of interest are their solubility in water ($S_w$, mg/L, at 20 °C) and in an organic solvent, frequently characterized by the logarithm of another partition coefficient ($K_{ow}$, pH = 7, 20 °C) between octanol and water. Their partition between air and water ($K_H$, Henry coefficient, $Pa/m^3/mol$) also indicates the extent to which they could volatilize from soil. Other characteristics like their molecular mass (M, g/mol), the number of substitute groups in pesticide molecule, those including halogen atoms, their ionization degree,… may also help understanding which processes they could undergo in the environment (Calvet et al., 2005a; FOOTPRINT, 2010).

Among these physico-chemical parameters, pesticide adsorption coefficients $K_{oc}$ are good indicators of pesticide aquatic fate (Watanabe et al., 2007). In order to provide a more comprehensive reading of the following parts, which deals with a very wide range of pesticide

molecules, pesticide $K_{oc}$ values will be provided. These values were extracted from the FOOTPRINT pesticide properties database (FOOTPRINT, 2010).

## 3 Buffer zones for pesticide pollution mitigation

A previous review was published assessing the efficiency of several mitigation measures for pesticide pollution reduction (Reichenberger et al., 2007) including conservation tillage practices, grassed waterways, vegetated buffer strips, constructed wetlands, vegetated ditches, forest buffers…

Among buffer zones, grassed buffer strips have been frequently documented (Muscutt et al., 1993; USDA-NRCS, 2000; Lacas et al., 2005; CORPEN, 2007). It was postulated that edge-of-field buffer strips are more efficient than riparian buffer strips for drift control. Grassed buffer strips have high permeability thus conferring them a high infiltration capacity by which pesticides are removed (Lacas et al., 2005; CORPEN, 2007). However, it was shown that for soil saturated conditions or large runoff events (concentrated flow, high velocity), the efficiency is significantly reduced due to decreased infiltration capacity (Souiller et al., 2002).

Vegetated ditches also demonstrated high potential for pesticide pollution reduction (Bennett et al., 2005). Pesticide association to sediments and ditch vegetation has been recognized as an important removal process (Cooper et al., 2002) attributed to adsorption (Margoum et al., 2006) and plant uptake (Bouldin et al., 2006). As for buffer strips, during large storm events, their efficiency in relation to runoff pollution is reduced but spray-drift mitigation was still recorded (Dabrowski et al., 2006).

### *3.1 Pesticide pollution reduction in forest buffers*

Forest buffers present organic matter rich soils where water level fluctuations generate alternate aerobic and anaerobic conditions enhancing nitrate removal by plant uptake and denitrification (Snyder et al., 1995; Ruffinoni, 1996; Lowrance et al., 1997a). Contrary to nutrient control, forest buffer potential to reduce pesticide concentrations and loads was part of a limited number of studies (Lowrance et al., 1997b; Vellidis et al., 2002; Gay et al., 2006; Pinho et al., 2008). Although they indicated high levels of pesticide reduction, it should be noted that the previous four studies were conducted in the same USA state (Georgia) and three of them focused on the same research site. In addition, they rely on riparian forest buffers which consist in a three-zone buffer: (i) a grassed strip near the field, (ii) a managed forest and (iii) an undisturbed forest adjacent to the stream bank. The whole buffer showed very high concentration reductions for atrazine ($K_{oc}$ = 100 mL/g) and alachlor ($K_{oc}$ = 124 mL/g) decreasing from 12.7 and 1.3 µg/L (inlet) to 0.66 and 0.06 µg/L (outlet), respectively, for a 38-m long buffer (Vellidis et al., 2002). Over a 50-m long distance, (Lowrance et al., 1997b) found that inlet concentrations of 34.1 (atrazine) and 9.1 (alachlor) µg/L were reduced to 1 or less than 1 µg/L at the system outlet. Increasing water flowpath therefore seemed to help decrease these moderately sorbing herbicides. Infiltration and degradation of atrazine were observed (Gay et al., 2006). They calculated removal rates ranging from 84 to 100 % for atrazine and three of its degradation products. However, they determined that the grassed portion of the whole buffer system accounted for the highest removal rates, followed by a 10-m wide pine area. For a 10-m long forest buffer, Pinho et al. (2008) observed atrazine and picloram ($K_{oc}$ = 35 mL/g) mass reduction by 47 and 28 %, respectively. They conducted additional laboratory experiments to assess these herbicides adsorption on forest organic soil. They concluded that atrazine was removed from water by 72 %, whereas picloram did not undergo any removal mechanism, probably due to its low sorption coefficient. Indeed, their field study also highlighted that very low (5 %) concentration reduction was found for picloram (and 28 % for atrazine) thus indicating that water infiltration accounted for most of

their load reduction. Groundwater contamination can therefore be feared. In addition, groundwater flow towards stream may contribute to pesticide pollution thus dampening the effect of riparian forest, as noted previously for atrazine (Angier et al., 2002). Other studies assessed herbicide mitigation in forest soils used for weed control in wood production areas (Veiga et al., 2001; Dousset et al., 2004; Newton et al., 2008). Veiga et al. (2001) found that glyphosate ($K_{oc}$ = 21699 mL/g) and its main degradation product AMPA ($K_{oc}$ = 8027 mL/g) were both degraded in forest soils planted with *Eucalyptus nitens*. Surprisingly, despite their high sorption coefficients, they were found to rapidly move below the first 30-cm horizon. Conversely, Dousset et al. (2004) observed low glyphosate migration through soils from Christmas tree production. Finally, Newton et al. (2008) concluded that glyphosate could be dissipated from forest soil from high altitude (cold climate) areas.

Once saturated, grassed buffer strip efficiency decreases (Souiller et al., 2002), whereas forest buffers could provide additional reduction of pollution thanks to their accumulated litter through which excess water can run off. The forest soil particulate organic matter was shown to provide high sorption of diflufenican ($K_{oc}$ = 3186 mL/g) and isoproturon ($K_{oc}$ = 122 mL/g) although isoproturon was found to easily desorb (Benoit et al., 2008). Leaves in decay, as found in vegetated ditches or forest buffers, were attributed a higher sorption potential than sediments for isoproturon, diflufenican and diuron ($K_{oc}$ = 1067 mL/g) (Margoum et al., 2006). Some studies showed that trees could absorb pesticides and further metabolize or store them (Karthikeyan et al., 2004). For instance, atrazine was uptaken by poplar trees up to 27.8 and 29.2 % after 52 and 80 days, respectively (Burken and Schnoor, 1997). Trees' rhizosphere is a favorable environment thanks to soil oxygenation, dead roots organic material and root exudates stimulating microbial activity (Burken and Schnoor, 1997; Karthikeyan et al., 2004).

### *3.2 Pesticide pollution reduction in artificial wetlands*

A review dealing with pesticide mitigation through artificial wetlands has been conducted through the ArtWET LIFE project (Gregoire et al., 2008). Previously, Schulz (2004) reviewed nine on-site constructed wetlands for insecticide pollution and Reichenberger et al. (2007) evaluated the efficiency of several mitigation measures including constructed wetlands for pesticide pollution control. A paper dealing with agricultural non point source pollution mitigation through restored or constructed wetland has just been released in 2010 and partly assessed pesticide pollution (O'Geen et al., 2010). Overall, despite wide variability, most artificial wetlands showed promising results in their potential to limit pesticide pollution since most of the efficiency measurements were close to or greater than 60% (Reichenberger et al., 2007). For instance, Schulz (2004) showed that insecticide reduction ranged from 54 to 99%, with most of the results exceeding 90%. More recently, Imfeld et al. (2009) published a paper focusing on constructed wetlands internal processes governing organic chemical fate. Removal processes include volatilization and phytovolatilization, plant uptake and phytoaccumulation, sorption, sedimentation of particle-bound molecules, phytodegradation (degradation by plant enzymes), microbial degradation and oxidation – reduction processes, oxidation being susceptible to be mediated by light (photodegradation) (Schulz et al., 2003a; Gregoire et al., 2008; Imfeld et al., 2009). Among degradation processes, microbiological removal of organic compounds appeared to predominate in constructed wetlands (Reddy and Dangelo, 1997; Hijosa-Valsero et al., 2010).

Among 23 identified papers specifically dealing with on-site field- or large mesocosm-scale constructed wetlands implemented to treat pesticide pollution, 13 were conducted in North America, 6 in Europe, 3 in South Africa and 1 in Australia (Appendix I). In addition, 10 were conducted under simulated conditions and 13 reported wetland efficiencies to reduce real runoff pesticide pollution. For instance, several papers assessed wetland efficiency based on artificial inlet concentrations sometimes unrealistically high: 3 to 733 µg/L (Moore et al.,

2000; Moore et al., 2001b; Moore et al., 2002; Moore et al., 2009a), 400 mg/L (Runes et al., 2003), 0.9 to 326 µg/L (Sherrard et al., 2004). There is therefore a need to monitor wetland systems under realistic conditions.

Pesticide transfer between wetland inlet and outlet can be retarded compared to water flows (Alvord and Kadlec, 1996; Kidmose et al., 2010). A wide range of efficiencies (mass or concentration reduction) is usually recorded (Appendix I): 15 to 41 % (Blankenberg et al., 2007), 26 to 33 % (Alvord and Kadlec, 1996), 0 to 100 % (Cheng et al., 2002), -10 to 95 % (Hunt et al., 2008), 0 to > 99 % (Matamoros et al., 2007; Matamoros et al., 2008a), -215 to 96 % (Miller et al., 2002), 34 to 70 % (Moore et al., 2000), 87 to 91 % (Moore et al., 2001b), > 90 % (Moore et al., 2002), > 80 % (Moore et al., 2009a), 0 to 55 % (Rose et al., 2008), 54.1 % (Schulz and Peall, 2001), 60.5 % (Schulz et al., 2003a), -11 to 67 % (Braskerud and Haarstad, 2003; Haarstad and Braskerud, 2005), 63.2 to 82.4 % (Stearman et al., 2003). This listing shows that wetlands could exhibit very promising results for pesticide pollution reduction. However, such variability indicates that their functioning is not yet totally understood or optimized.

Indeed, "negative efficiencies" were noted in some cases, signs of higher concentrations at the outlet than at the inlet (Miller et al., 2002; Runes et al., 2003; Haarstad and Braskerud, 2005; Rose et al., 2006; Hunt et al., 2008). It is important to note that, particularly for studies conducted under realistic conditions, pesticide concentrations can be close to the analytical limit of quantification (Miller et al., 2002). Efficiencies based on such values, where uncertainties are usually the highest, may lead to unreliable results. Biofilm detachment, onto which pesticides could have sorbed, or desorption from biofilms, sediments or other substrates, may explain remobilization of pesticides and higher outlet than inlet concentrations (Headley et al., 1998). Hydrophobic pesticides attached to suspended particles can be removed through their sedimentation (Schulz, 2004; Skagen et al., 2008), whereas weakly sorbing molecule concentrations can be reduced through dilution and diffusion (spread out) in the reservoir (Itagaki et al., 2000).

The presence of nitrogen atoms in pesticide molecules have been suggested to improve pesticide degradation (Kao et al., 2001; Braskerud and Haarstad, 2003). It has been noted in some occasions that higher influent concentrations were associated with higher concentration reductions (Haarstad and Braskerud, 2005). A seasonal trend in pesticide removal can be observed (Matamoros et al., 2008b). Warmer temperatures improve microbial activity leading to higher pesticide removal rates (Lartiges and Garrigues, 1995); however, other factors like plants, pH and oxygen could also affect pesticide removal (Machate et al., 1997). Haarstad and Braskerud (2005) pointed out that sometimes, despite some concentration reduction (-18 to 67 %) in wetlands, outflow concentrations could be harmful to aquatic life. Both mass and concentration reductions should be targeted for an environmental protection perspective. In addition, not only pesticide mass or concentration "percentage reduction" are important, but their outflow values as well. Wetland efficiency is closely related to pesticide physicochemical characteristics, particularly concerning sorption and degradation processes. Few data were gathered for moderately and weakly sorbing molecules (Moore et al., 2000; Braskerud and Haarstad, 2003; Reichenberger et al., 2007). In addition, most studies dealing with wetlands efficiency to reduce pesticide pollution focused on “old” molecules, released in the market between 1950 and 1980 (Fig. 2).

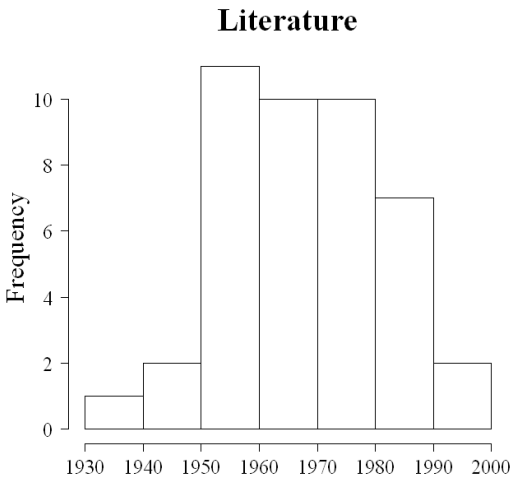



**Fig. 2: Histogram on pesticide introductory dates on the market for molecules belonging to published literature.**

Very few studies pointed out the relevance of wetland system efficiency with respect to overall watershed outlet pollution. Indeed, as previously pointed out, many studies were based on simulated polluted flows for which this parameter is not applicable. Haarstad and Braskerud (2005) provided results about the portion of applied pesticides on watershed that was reduced in constructed wetlands (0.3 to 9.4 % for 7 pesticides). However, the key parameter should deal with the portion of watershed outlet pesticide loads caught by and reduced through wetlands to estimate system efficiency. Indeed, unless having large enough surface area to create a wetland large enough to catch all watershed outflows ("instream" wetland), a system would bypass through the wetland only a portion (X %) of catchment outflow ("offstream" or "in parallel" wetland). The complement of watershed outflow (100−X %) directly going to receiving waters will not undergo any treatment. Consequently, even if the wetland can reduce Y = 100 % of total incoming flows (X %), only X×(1−(100−Y)/100) % of watershed outflows would have been treated.

Small wetlands that could not accommodate all water volumes generated by the watershed should focus on watershed flows presenting the highest risk of pesticide transfer. An important part of the work should therefore focus on acquiring knowledge about pesticide transfer dynamics in agricultural watershed. This will help determining which flows show the highest concentrations and loads. Such an approach has not been documented whereas it is critical particularly in Europe where agricultural land-availability may be a major constraint.

*3.3 Nitrate pollution reduction in artificial wetlands*

Non point source agricultural pollution not only comprises pesticide, but nitrate pollution as well. Wetland efficiency at reducing nitrate pollution has been extensively studied. Both natural (Lowrance et al., 1995; Fisher and Acreman, 2004) and constructed (or artificial) (Vymazal et al., 2006; Kadlec, 2009) (CW) wetlands have been widely assessed for their ability to remove nitrate from agricultural (Braskerud, 2002; Tanner et al., 2005), municipal and industrial wastewaters (Hammer, 1989; Vymazal, 2005; 2009). Constructed wetlands, particularly those implemented for wastewater treatment, have often been classified according to vegetation type (floating vs. emergent) and water flow regime (free water surface (FWS), horizontal sub-surface flow (HSSF) or vertical flow (VF) wetlands (Kadlec and Wallace, 2008). This range of design types reflects the existence of multiple hydrological functions that have different effects on nitrogen cycling processes. Denitrification requires the presence of nitrate, a carbon source, limited oxygen concentration and denitrifying microorganisms. Denitrification is often regarded as the major nitrate transformation

mechanism in wetlands (Reddy and Patrick, 1984; Bachand and Horne, 2000; Tanner et al., 2005). Vymazal et al. (2006) reported a very wide range of published denitrification rates spanning three orders of magnitude between 0.003 and 1.02 $gN.m^{-2}.yr^{-1}$. However, plant uptake and subsequent harvesting may also be an important process to remove nitrate, with removal rates approaching 600 $gN.m^{-2}.yr^{-1}$.

Studies of nitrate removal in both natural and constructed wetlands have demonstrated a large range of efficiencies. Kadlec (1994) found nitrate removal efficiencies between -138 and 96% for 11 natural and 13 constructed wetlands treating municipal or industrial wastewaters (influent $NO_3^-+NO_2^-$-N concentration range was 0.1-18.0 mg/L). A similarly wide range [1-100%] was reported by Fisher and Acreman (2004) in a review of 20 natural wetlands receiving nitrate from different sources (agricultural or sewage effluents, subsurface runoff). In a study of five tropical wetlands receiving low influent nitrate concentrations (lower than 1 mgN/L) from a dairy farm, a dairy processing plant, a banana paper plant and a landfill, Nahlik and Mitsch (2006) found between -207% and 89% nitrate removal. Nitrate removal rates in constructed wetlands are often compared to rates measured in natural wetlands. Hammer and Knight (1994) found a 44% average efficiency for 17 FWS constructed wetlands and a 77% efficiency for 26 natural wetlands. For an additional nine sub-surface flow CW with high (over 100 mg/L) influent nitrate concentrations, nitrate retention was 9%. Technical reports, such as the NCDENR BMP manual, indicate a 40% overall nitrogen reduction in stormwater wetlands (NCDENR, 2005). We reviewed over 16 individual studies on nitrate retention in wetlands to compare performance in constructed and natural wetlands treating effluents from various sources (Appendix II). Papers presenting available data on nitrate removal efficiencies were selected. The results of this review were consistent with the studies cited above, with average concentration-based nitrate removal percentages of 44% for CW and 73% for natural systems. On a mass-basis, nitrate removal efficiencies were 46% (CW) and 69% (natural wetlands). Consequently, it appears that natural systems typically demonstrate higher nitrate retention than CW. Although they are less efficient, CW on average do reduce inlet nitrate concentrations or loads by at least 40%.

There are several explanations for the higher efficiencies reported in natural wetlands. It was generally found that higher efficiencies were associated with vegetated or more densely vegetated wetlands, compared to unvegetated or less densely vegetated wetlands (Kadlec, 2005; Tanner et al., 2005; Bastviken et al., 2009). For CW, it has been suggested that planting mixed vegetation could promote higher denitrification rates than one-species stands (Bachand and Horne, 2000). Natural wetlands contain vegetation that has had a longer period to establish than most studied constructed wetlands. The former therefore have had more time to accumulate soil organic carbon than the latter (Craft, 1997; Appelboom and Fouss, 2006). Denitrifying microorganisms in natural wetlands are therefore less likely to be carbon limited. Increases in carbon content have been shown to improve soil denitrification capacity provided other factors are not limiting (Reddy and Patrick, 1984). Baker (1998) suggested that the C:N ratio for nitrate removal be at least 5:1 to prevent carbon limitation. Soil reducing conditions also need time to establish. Consequently, during the first few years, denitrification in CW may be limited either by the absence of reduced conditions or available carbon or both (Craft, 1997). A comparative study between HSSF and FWS systems showed better performance for denitrification for HSSF systems (Kadlec, 2009). This was attributed to the HSSF configuration supporting more anaerobic conditions thus promoting the development of denitrifer populations that transform nitrate to gaseous nitrogen forms. However, it is important to note that denitrification was also observed before oxygen was completely depleted (Phipps and Crumpton, 1994; Vymazal et al., 2006). Bachand and Horne (2000) showed that surface water dissolved oxygen did not affect denitrification rates. As for pesticide removal, in some cases, "negative efficiencies" were reported for nitrate reduction (Kadlec, 1994; Nahlik and Mitsch, 2006; Knox et al., 2008). Ammonia transformation to

nitrate or organic matter mineralization can occur within the wetland system, particularly in the most aerated FWS wetlands (Kadlec, 2009). This may lead to net increases in nitrate concentrations or loads between the inlet and the outlet of wetlands.

Several other factors are also known to affect nitrate removal efficiencies. Nitrate losses were shown to increase with increasing temperature (Kadlec, 2005; Beutel et al., 2009). The optimal temperature for denitrification was observed to fall in the range of 60 to 75 °C (Reddy and Patrick, 1984). However, high denitrification rates did occur between 5 and 60 °C and lower denitrifying activity could be measured below 4°C (Vymazal et al., 2006). A seasonal nitrate removal pattern was generally observed, with higher nitrate removal efficiencies during the warmest and wettest seasons (summer) (Christensen and Sorensen, 1986; Bachand and Horne, 2000; Spieles and Mitsch, 2000; Richardson et al., 2004; Beutel et al., 2009). Hydraulic factors may also impact nitrate reduction. Large flood events induce lower retention times and higher nitrate loading producing poor nitrate retention (Spieles and Mitsch, 2000). In addition, several studies found lower nitrate concentration reduction for higher hydraulic loads (Baker, 1998; Spieles and Mitsch, 2000; Kadlec, 2005; Bastviken et al., 2009). Finally, Bachand and Horne (2000) found that neither influent nitrate concentrations (average was 9.27 mg $NO_3^-$-N/L) nor surface water dissolved oxygen affected removal rates in FWS constructed wetlands; whereas, organic carbon availability and water temperature did.

## 4 Key parameters for artificial wetlands

Wetland main three compartments (water, sediments and plants) characteristics affect pollutant removal mechanisms and efficiencies. A brief overview of the influence of each of these compartments is given below.

### *4.1 Water*

Wetland hydrologic properties affect transport of contaminant through the system. Deep wetland may cause larger dilution, slower flows and lower sediment resuspension which may enhance sediment-bound pesticide removal (Budd et al., 2009). Contact time between pesticides and microbial population growth surfaces is determining in pesticide degradation. Consequently, a key parameter in treatment wetlands consists in the duration water, and pesticides, remain in the system. This is often characterized by the hydraulic retention times ($\tau$) determined experimentally through tracer tests. A first approximation of this parameter is the nominal detention time ($T_n$) calculated dividing the wetland volume by the average flow rate (Kadlec and Wallace, 2008). It was demonstrated previously that the higher the retention time, the higher the removal rates (Alvord and Kadlec, 1996; Runes et al., 2003; Stearman et al., 2003; Conkle et al., 2008; Llorens et al., 2009). Increase in electrical conductivity may also help decreasing pesticide concentrations (Braskerud and Haarstad, 2003; Haarstad and Braskerud, 2005) which may be explained by increased flocculation and sedimentation of particles.

### *4.2 Vegetation*

The presence of vegetation helps decreasing concentrations of organic compounds through direct and indirect effects (Stearman et al., 2003; Stottmeister et al., 2003; Slemp et al., 2004; Rogers and Stringfellow, 2009; Hijosa-Valsero et al., 2010). Schulz et al. (2003b) studied linear (0 to 40 m) and temporal (3 h to 10 d) evolution of parathion methyl ($K_{oc}$ = 240 mL/g) concentrations through vegetated and non vegetated wetland cells (50 × 5.5 × 0.2 m). Ten days after the injection of a high (400 mg/L) concentration in parathion methyl, outlet concentrations at 40 m were 0.6 and <0.1 µg/L for the non-vegetated (plant coverage lower than 5 %) and vegetated (90 % plant coverage, mostly *Juncus effusus*) wetlands, respectively. Concentration decrease in the vegetated wetland was faster and more pronounced than in the non-vegetated wetland. *Elodea densa* managed to accumulate chlorpyriphos ($K_{oc}$ = 8151

mL/g, ) up to 64 % (Karen et al., 1998) and isoproturon (Feurtet-Mazel et al., 1996; Merlin et al., 2002). Feurtet-Mazel (1996) showed that isoproturon bioaccumulation in *Elodea densa* and *Ludwigia natans* was rapid and more important in the 0 – 100 µg/L concentration range in water than in the 10-fold higher concentration range thus indicating saturation. For five plant species (*L. minor, S. polyrhiza, C. aquatica, C. palustris and E. canadensis*), lower removal rates were found for two fungicides, dimethomorph ($K_{oc}$ = 348 mL/g; 10 to 18 %) and pyrimethanil ($K_{oc}$ = 301 mL/g; 7 to 12 %) (Dosnon-Olette et al., 2009). Bulrush (*Schoenoplectus californicus*) roots were shown to uptake the highest concentrations of organochlorine pesticides, mainly from the sediments compartment (Miglioranza et al., 2004). These authors found that plant stems from the water column appeared to significantly remove the least hydrophobic molecules. It seems that hydrophobic compounds are more easily associated with plants than hydrophilic molecules, as previously reported for organic chemicals (Imfeld et al., 2009). However, while assessing the effect of four plant species on permethrin ($K_{oc}$ = 100000 mL/g) removal from wetland mesocosms, Moore et al. (2009b) found that between 77 and 95 % of permethrin mass was associated with sediments for three (*L. oryzoides, T. latifolia and T. dealbata*) of the four studied plant species. This may be due to the insecticide extremely high sorption coefficient. In another study conducted in a constructed wetland (180 × 30 m), Moore et al. (2007) observed that 43 % of diazinon ($K_{oc}$ = 643 mL/g) mass was measured in plant materials, whereas, only 23 % was found in sediments. It was also noted that 10 % of metolachlor ($K_{oc}$ = 200 mL/g), 25 % of chlorpyrifos, 76 % of cyfluthrin ($K_{oc}$ = 64300 mL/g) and 81 % of methyl-parathion ($K_{oc}$ = 240 mL/g) masses were found in plants (Moore et al., 2001b; Moore et al., 2002; Moore et al., 2006; Moore et al., 2009a). Two studies from the same researcher team found varied results for lambda-cyhalothrin ($K_{oc}$ = 157000 mL/g) in plants: 49 % (Moore et al., 2009a) and 87 % (Moore et al., 2001a) indicating some variability between studies. Contrary to Moore et al. (2000) who did not quantify atrazine ($K_{oc}$ = 100 mL/g) in wetland plants, Cejudo-Espinosa et al. (2009) reported atrazine accumulation in plant roots (40 %) and demonstrated that pesticide uptake by wetland vegetation is dependent on atrazine concentration and plant species. Lee et al. (1995) compared the effect of three wetland mesocosms hydrophyte communities including no-vegetation, submerged and emergent hydrophytes, on atrazine and alachlor degradation. They concluded that alachlor was more rapidly degraded than atrazine whatever the vegetation structure; whereas atrazine degradation rate was the highest in the emergent hydrophytes wetland mesocosms. Pesticide molecules hydrophobicity or sorption capacity therefore appears to partly govern plant-associated pesticide mass. Indeed, as previously indicated, strongly sorbing molecules would be frequently found in plant materials; however, this effect may be limited for extremely sorbing pesticides for which a rapid adsorption to bottom sediments may occur before being in contact with wetland plants. Interestingly, through a laboratory experiment, Friesen-Pankratz et al. (2003) observed that algae (*Selenastrum capricornutum*) decreased atrazine and lindane ($K_{oc}$ = 1100 mL/g) persistence thus possibly facilitating their sorption or degradation as also noticed for fluometuron ($K_{oc}$ = 67.4 mL/g), aldicarb ($K_{oc}$ = 30 mL/g), diuron, endosulfan ($K_{oc}$ = 11500 mL/g). Pesticide sorption enhancement by algae was also suggested (Friesen-Pankratz et al., 2003; Rose et al., 2006). Finally, negative effect of pesticides on plant should also be considered. For instance, several test were carried out to assess metazachlor ($K_{oc}$ = 134 mL/g) effect to macrophytes (*Potamogeton natans, Myriophyllum verticillatum, Cladophora glomerata*) and phytoplankton communities with inlet concentrations ranging from 5 to 500 µg/L (Mohr et al., 2007; Mohr et al., 2008). They concluded that single exposure to metazachlor concentrations larger than 5 µg/L could affect ecosystem function and aquatic biota.

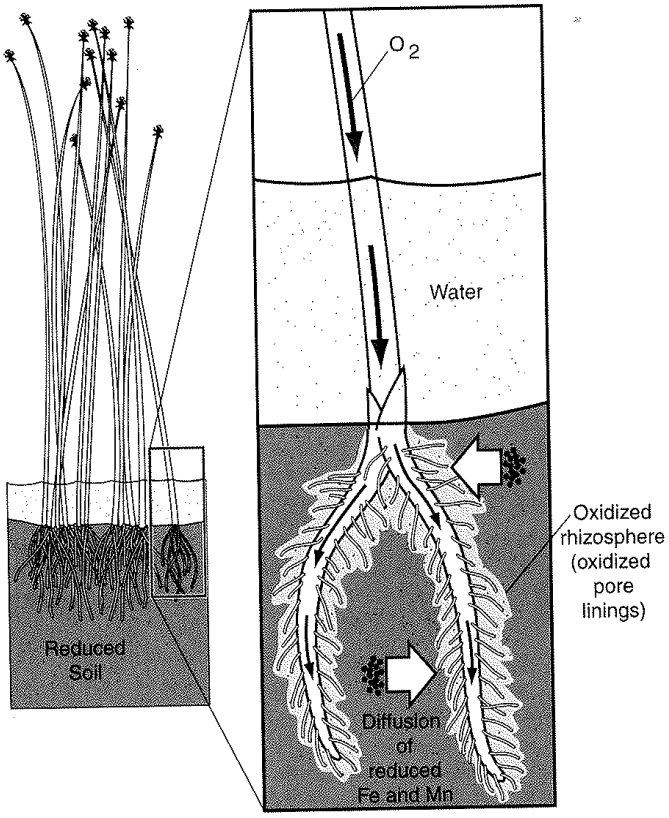



**Fig. 3: Oxido-reduction states of a wetland soil and wetland plant rhizosphere (after Mitsch and Gosselink, 2000).**

Wetland plant vegetation does not only affect pesticide fate through such direct adsorption or absorption processes. Indeed, a series of indirect effects significantly contributes to pesticide mitigation. For instance, plants help soil aeration thus supporting microbial activity (Hammer, 1992; Headley et al., 1998; Luckeydoo et al., 2002; Susarla et al., 2002). As previously noted, this effect generates oxygen concentration gradients in wetland soils into which both aerobic and anaerobic processes can take place (Mitsch and Gosselink, 2000) (Fig. 3). The presence of vegetation creates roughness decreasing flow velocity and increasing particle sedimentation (Rose et al., 2008) thus playing a role of physical filtration (Hammer, 1992; Brix, 1997). Plant organic matter helps pesticides transferring from water to plant material (Moore et al., 2007). In addition, macrophyte surface area enable microbial attachment and biofilm development into which degradation reactions can take place (Hammer, 1992; Brix, 1997; Rose et al., 2008). They also provide bed sediments stabilization (Brix, 1997). It was previously shown that plants may support microbial activity in the root zone because of increased soil aeration (Susarla et al., 2002). Rose et al. (2008) showed that vegetated systems improved photolysis, which is counter-intuitive with the fact that high plant coverage implies increased shading. They suggested that shading negative effect on photodegradation was counterbalanced by increased water clarity and light penetration due to suspended particle sedimentation.

| Vegetation tissue | Role in artificial wetland |
|---|---|
| Plant tissues in the air (leaves) | - Increase shade,<br>- reduce wind speed,<br>- insulate water from freezing,<br>- aesthetic aspects. |
| Plant tissues in contact with water | - Adsorption (and desorption) sites,<br>- support for microbial growth,<br>- roughness reducing water velocity and increasing sedimentation,<br>- excretion of photosynthetic oxygen. |
| Roots and rhizomes in sediments | - Excretion of photosynthetic oxygen,<br>- up-take of some pesticide molecules,<br>- sediment stabilization. |

**Table 1: Artificial wetland vegetation role for pesticide pollution mitigation, inspired from Brix (1997).**

*4.3 Sediments*

The importance of wetland soil is manifold: it is a support for plants and presents reactive surfaces where pollutants can attach or micro-organisms can grow (Hammer, 1992). Water level fluctuation has an influence on soil aeration and consequently on redox potentials leading to the alternation between flooded (anoxic or anaerobic) and non-saturated (aerobic) conditions (Reddy and Patrick, 1984; Faulkner and Richardson, 1989; Tanner et al., 2005). A large variation in oxygen concentrations was measured between surface waters and soil layers as well as within the plant-root system itself (Brix, 1987; Christensen et al., 1989; Reddy et al., 1989) (Fig. 3). Under flooded conditions, oxygen is more rapidly depleted through microbial activity consumption than it is transferred from atmosphere through diffusion (Faulkner and Richardson, 1989). Indeed, oxygen transfer in water-logged soil is 10000 times slower than in gas-filled pores (Ponnamperuma, 1972). During the first days of soil submersion, pH decreases before increasing again to a fairly steady value around 7 (Ponnamperuma, 1972). However, for natural wetlands with low outflow rates and poor-nutrient content like bogs, pH is usually acidic (Mitsch and Gosselink, 2000). Microorganisms mediating oxidation reactions (like organic matter decomposition) require energy they can retrieve from oxidant (electron acceptor) species. After depletion of the most energetic oxidant (oxygen), nitrate, manganese, iron, sulphur and carbon dioxide will be successively used due to their decreasing energy contribution by several microorganisms (McBride, 1994; Kadlec and Wallace, 2008). The oxidation state of a soil is determined by its redox potential at equilibrium ($E_h$, mV) usually measured by means of platinium electrodes (Patrick et al., 1996). It represents the electron availability, i.e., the tendency of a system to oxidize or reduce chemical species (Bohn, 1971). Wetland soil redox potentials range from -400 (strongly reduced) to +700 (well oxidized) mV (Kadlec and Wallace, 2008) and oxygen disappears under approximately +350 mV (McBride, 1994) (Fig. 4).

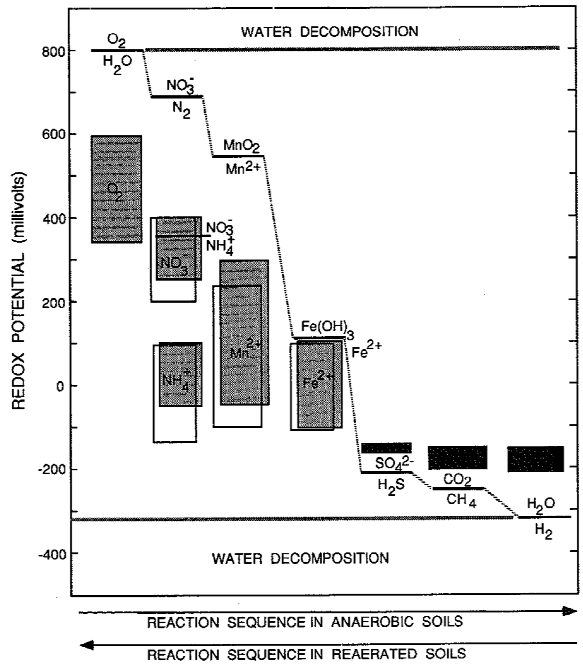



**Fig. 4: Reduction and oxidation sequence in soil solution at pH 7 (from McBride, 1994).**

Wetlands comprise plant species that are usually able to transport oxygen from above-ground stems and leaves to below-ground roots. This results in a thin oxidized layer around plant roots (rhizosphere) where aerobic reactions can take place (Hammer, 1992; Mitsch and Gosselink, 2000). Consequently, a wide range of redox conditions can be found in wetland soils along time (according to water level induced flooded conditions) and space (depending on proximity to plant stems).

The effect of redox conditions on dissolved substances was studied for a long time starting by focusing on simple chemical species like nitrate, sulphate, iron, manganese, carbon dioxide, ammonium (Pearsall and Mortimer, 1939; Mortimer, 1942; Dusek et al., 2008) to more complex ones including pesticides, more recently (DeLaune et al., 1997; Reddy and Dangelo, 1997; Charnay et al., 2000; Seybold et al., 2001; Weaver et al., 2004; Accinelli et al., 2005; Lu et al., 2006; Laabs et al., 2007). Hijosa-Valsero et al. (2010) found that oxygen concentration and redox potential showed either positive or negative correlation with pharmaceuticals and personal care products removal from mesocosm constructed wetlands. Pesticides can act as oxidants in reducing environments (Calvet et al., 2005a). These authors summarized which functional substitute groups may undergo reduction reactions (nitro aromatic, sulfoxyde and alkyl groups) and those which are not susceptible to be reduced (aldehydes, ketones, carboxylic and ester groups, amides). Anaerobic dehalogenation of pesticides was also studied (Kuhn and Suflita, 1987). Charnay et al. (2000) showed that reducing conditions slightly enhanced atrazine and isoproturon adsorption to wetland soils and lead to a larger fraction of non-extractable residues for atrazine but not for isoproturon. None of the herbicides was mineralized under low redox conditions and degradation was found to be significantly slowed down. Other studies on wetland sediments confirmed reduced or inexistent mineralization of atrazine (DeLaune et al., 1997; Larsen et al., 2001; Weaver et al., 2004), isoproturon, metsulfuron-methyl ($K_{oc}$ = 39.5 mL/g), mecoprop ($K_{oc}$ = 31 mL/g) and fluometuron (Larsen et al., 2001; Weaver et al., 2004) under anaerobic conditions. However, Chung et al. (1996) partly explained the observed atrazine concentration decrease by possible mineralization, despite not being able to show any evidence of it. Kao et al. (2001) found that atrazine could be removed under anaerobic conditions provided sucrose is added. In addition, they found that atrazine can serve as a nitrogen source for microorganisms

under reducing conditions. Under aerobic conditions, Anderson et al. (2002) observed high atrazine mineralization rates (70–80 %) in the first 5 cm of wetland sediments. Consequently, when returning to un-flooded (aerated) conditions, wetland sediments seem to exhibit high potential for pesticide removal. Another study showed that flooding soils increased persistence of isoproturon and linuron ($K_{oc}$ = 620 mL/g). However, metolachlor half-life decreased from 32.2 (non-flooded) to 24.1 (flooded) days while redox potential sharply decreased from +368 to -225.5 mV in the first week of incubation (Accinelli et al., 2005). This is not in accordance with other water/sediment laboratory results obtained by Hoyos-Hernandez (2010) on S-metolachlor dissipation. Besides being a support for microbial growth and associated pesticide degradation, wetland sediments can also adsorb pesticides (Runes et al., 2003). For instance, up to 55 % for chlorpyrifos (Moore et al., 2002) and 28 % for fipronil ($K_{oc}$ = 577 mL/g) (Peret et al., 2010) were adsorbed onto sediments. It is however important to note that pesticide adsorption to sediments is site specific.

## 5 Design guidelines for water treatment objectives

### *5.1 Ecological engineering principles: application to buffer zones*

The concept of buffer zones is derived from the implementation of basic principles of "ecological engineering", also called "eco-technology" which combines ecosystem functions and human needs. "Ecological engineering combines basic and applied science for the restoration, design and construction of ecosystems including wetlands" (Mitsch, 1992). It includes the development of new ecosystems or the restoration of ecosystems previously disturbed by human activities. Mitsch (1992) listed ecological engineering principles that could be applied to wetland creation. Wetland systems should be designed: with the landscape, for minimum maintenance, so as they use natural energies, for multiple objectives but keeping one major objective as a starting point, for function. Ecological engineering key principle is to avoid over-engineering and human intervention by giving the system time to self-design and self-organize.

### *5.2 Wetland design guidelines*

Constructed wetlands for a water quality improvement perspective mainly focus on wastewaters and target nutrients or organic matter compounds. There is a need to multiply on-site wetland demonstration projects for pesticide pollution treatment to derive design guidelines (van der Valk and Jolly, 1992; O'Geen et al., 2010). This was the main objective of the ArtWET LIFE project.

Major existing design principles were first set to improve wastewater pollution and mainly focused on nutrients or large organic matter loads. However, it is likely that wetlands designed for wastewater treatment may have common key design parameters with wetlands aiming at improving agricultural pesticide pollution. Among them, high residence time, low water flow velocities and the presence of substrates for adsorption and microbial growth can be highlighted.

Constructed wetlands can be classified according to their hydrological regime, surface flow (SW) or horizontal and vertical subsurface flow (HSSF and VSSF) wetlands (Fig. 5).

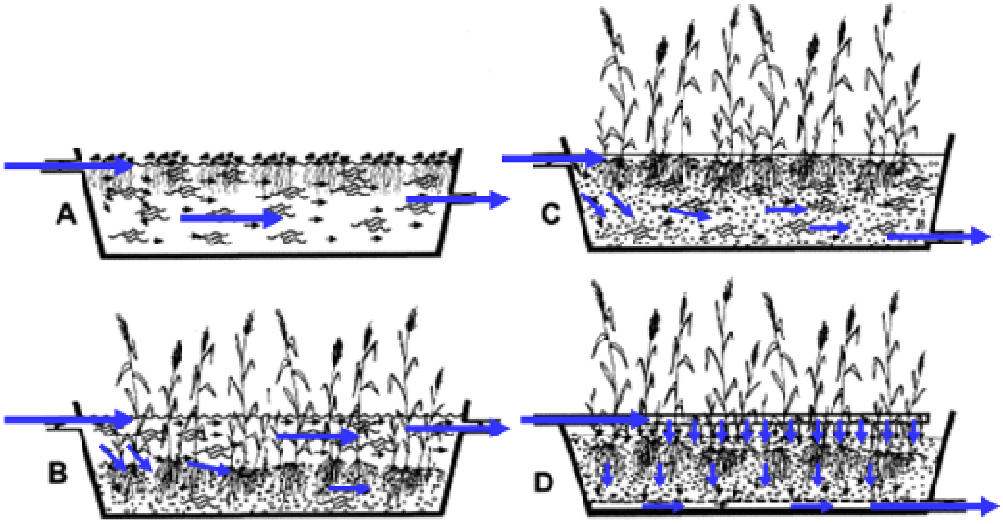



**Fig. 5: Constructed wetland systems for wastewater treatment (A: pond with floating plants; B: horizontal surface flow wetland or pond with emergent water plants; C: horizontal subsurface flow wetland; D: vertical flow wetland) (after Stottmeister et al. (2003)).**

| Wetland characteristics | Constructed wetlands | | Natural wetland |
|---|---|---|---|
| | FWS | SSF | |
| Min. area (ha, for 1000 m3/d) | 2 - 4 | 1.2 - 1.7 | 5 - 10 |
| Hydraulic load (m/d) | 0.025 – 0.05 | 0.058 – 0.083 | 0.01 – 0.02 |
| Max. water level elevation | 50 cm | Ground surface | Depends on vegetation |
| Max depth | | 30 – 90 cm | |
| Residence time (d) | 5 - 10 | 5 - 10 | 10 - 15 |
| Max load ($kg.ha^{-1}.d^{-1}$) | | | |
| DBO5 | 100 – 110 | 80 – 120 | 4 |
| SS | < 150 | | |
| Tot N | 10 – 60 | 10 – 60 ( >15) | 3 |
| Tot P | | | 0,3 – 0,4 |

**Table 2: Design guidelines for constructed wetlands treating wastewater pollution (after Girard et al. (2005)).**

Typical design guidelines imply constructed wetlands with 0.3 to 0.9 m deep water levels (Table 2). It is highly recommended to introduce vegetation like cattail (*Typha*), bulrush (*Scirpus*), reed (*Phragmites*), rushes (*Juncus*), submerged pondweeds (*Elodea, Potamogeton*)… (Hammer, 1992). To avoid short-circuits, it is preferable to plant banded versus fringing vegetation (Jenkins and Greenway, 2005). It is advised that the first part of the system be wide-shaped to slow down inlet flows, increase sedimentation, and facilitate flow distribution in the following parts. Length-to-width ratios of 3–5:1 are recommended (Hammer, 1992). Pollutant removal processes generally take place in the first parts of wetland systems (Machate et al., 1997; Matamoros et al., 2008b). In steep terrain, several wetlands could be constructed in a series of "terraced" wetlands (Mitsch, 1992) (Fig. 6). The inclusion of dikes can lengthen flow path thus increasing water and pollutant residence time in the system. Downward infiltration to groundwater may be prevented for soils whose hydraulic conductivity is lower than $10^{-6}$ cm/s (Hammer, 1992). Engineered wetland systems with added materials for microbial growth support and pesticide adsorption demonstrated their efficiency (Machate et al., 1997; Blankenberg et al., 2007; Matamoros et al., 2007).

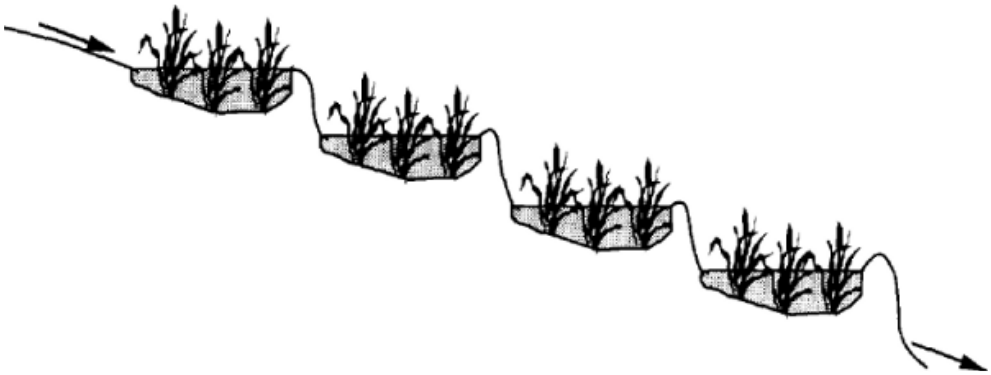


**Fig. 6: Terrace design for constructed wetlands in steep terrain (after (Mitsch, 1992)).**

Apart from these common parameters, others may be specific to pesticides whose physico-chemical properties bring increased complexity to optimize system functioning. In 1992, van der Valk and Jolly (1992) pointed out that the lack of information about pesticide fate in wetlands made it impossible to propose design guidelines for constructed wetlands for non point source pesticide pollution. In the same year, Rodgers and Dunn (1992) proposed a series of research questions to be tackled to better understand these systems and be able to develop design criteria. Jointly with a team of researcher in Mississippi between 2000 and 2002 (Moore et al., 2000; Moore et al., 2001b; Moore et al., 2002; Cullum et al., 2006), they proposed deriving wetland length from a set of basic equations assuming a pseudo-first order model for pesticide transfer and transformation processes. This simple modelling approach is described below. The basic principle is that pesticide concentration decrease according to a pseudo-first order model:

$$C_t = C_0 e^{-Kt} \quad \text{(Eq. 1)}$$

where $C_t$ and $C_0$ are pesticide concentration at time t and the initial concentration, respectively, and $K$ is and overall transfer and transformation rate coefficient ($t^{-1}$). Then, the Moore et al.'s researcher team used on-site monitoring data to get the duration needed to decrease pesticide concentration by half ($T_{1/2}$ for $C_t = \frac{C_0}{2}$) and then calculate $K$, expressed as $\frac{\ln 2}{T_{1/2}}$. Subsequently, given a desired percent removal $\frac{C_t}{C_0}$(%), wetland length ($d$, m) was derived from the following equation:

$$\frac{C_t}{C_0} = 100\% \cdot e^{-K \times d} \quad \text{(Eq. 2)}$$

From a series of studies monitoring pesticide concentration decrease along a wetland length and along time, they concluded on the need of a 100- to 280- m long wetland flow path to achieve significant atrazine reduction. Target outflow concentration in wetland design could be based on pesticide NOEC (no effect concentration) for aquatic invertebrates (e.g. *Daphnia magna*). Using these values, wetland lengths can be roughly estimated with regression equations (Moore et al., 2009a). Comparing vegetated versus non-vegetated wetlands (50 × 10 m), Moore et al. (2006) reaffirmed the importance of vegetation in treatment wetland systems. They suggested that an 18.8-m long vegetated wetland could decrease methyl-parathion concentrations to 0.1 % of its inflow concentration (8.01 mg/L), whereas the same objective could only be reached with a wetland length of 62.9 m if not including plants. Wetland lengths of 217 and 210 m were proposed as initial estimates to remove lambda-cyhalothrin and cyfluthrin, respectively.

Defining the correct wetland design and size is therefore crucial but still needs further on-site wetland research to harmonize design guidelines. Wetland efficiency may also decrease for small sized wetlands with low water storage capacities (Schulz and Peall, 2001).

Increasing size could therefore help improving treatment efficiency. For instance, Haarstad and Braskerud (2005) showed that increasing wetland size from 0.2 to 0.4 % of the watershed surface area increased average pesticide removal by 9 to 21 % units on average. However, one of the major issues on agricultural watershed relates to land availability for wetland creation. Today, despite an increasing number of research projects focusing on wetlands and pesticide pollution, O'Geen et al. (2010) conclude that more research linking pesticide removal of various physical properties with constructed wetland sizes, hydraulic residence time, flow and vegetation density is still needed to derive design guidelines.

*5.3 Location in the landscape*

In an agricultural context, the main stakeholders are farmers who generate pollution and own the land onto which buffer zone implementation can take place. Convincing them to concede a part of their cropped land to set up a buffer zone may be challenging. Social and economical considerations may be determining factors enabling or preventing wetland construction to treat agricultural non point source pollution. Such questions should be addressed to ensure the acceptance of treatment wetland implementation projects (van der Valk and Jolly, 1992).

The use of natural wetlands was not recommended (van der Valk and Jolly, 1992) as pollutant may have negative impacts on natural wetland flora and fauna which may comprise protected species. A good knowledge of landscape including watershed boundary, land-use, topography, input water pathway, is required to first identify elements that could be used as buffer systems like riparian non-cropped areas or forest areas. The location of buffer zones in watersheds is also important for their efficiency. Buffer zone effectiveness is usually higher when located at the outlet of small watersheds (Fig. 7). Indeed, concentrations are less diluted than for larger watersheds (Mitsch, 1992; CORPEN, 2007). Smaller upstream wetlands may also be a better strategy to survive extreme events (Mitsch, 1992). Less erosion and runoff would occur on the highest part than on the lowest part of a watershed. This could help reducing total wetland area needed by creating smaller individual wetlands higher in the catchment compared to constructing one large wetland at the catchment outlet (van der Valk and Jolly, 1992). However, it has been suggested that larger downstream wetlands may be more useful for flood control than multiple upstream systems (Mitsch, 1992). Finally, because of limited land-availability, it may not be possible to collect and treat all water volumes through constructed wetlands. Wetlands can either be instream- or offstream- (parallel) placed, and by-pass structures should be set-up to prevent over-flooding in case of large storm events.

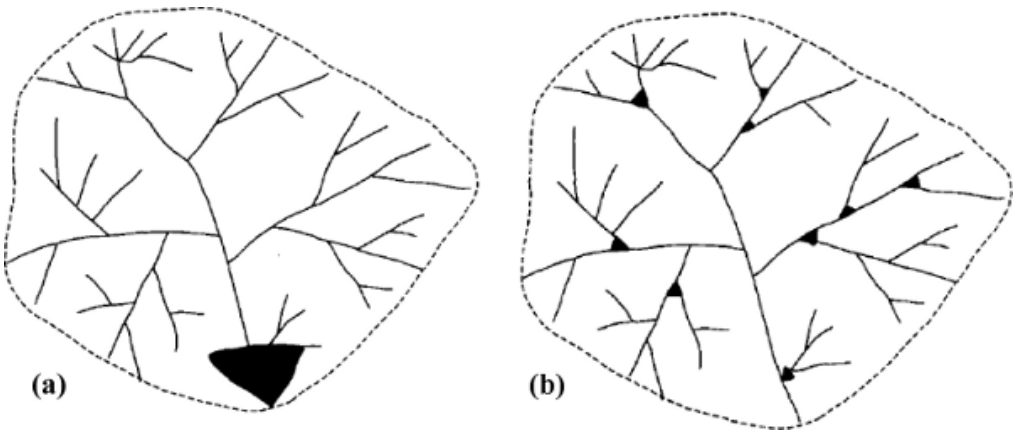



**Fig. 7: Possible location of wetlands in a landscape. (a) Basal model: one large wetland placed at the lower reaches of the watershed so that all water leaving the watershed passes through it. (b) Distributed model: a wetland placed at the lower reaches of each sub-watershed leading to reduced overall movement of water and contaminants within the watershed (after van der Valk and Jolly (1992)).**

## 6 Summary of remaining scientific questions

This review helped us derive a few questions that still need to be addressed about artificial wetlands treating pesticide contaminated flows. This Ph.D project attempted to provide some clues to partially answer these questions. We aimed at better understanding wetland functioning for non point source agricultural pollution in artificially drained watersheds.

- Few data have been collected about wetlands created to specifically treat artificially drained watershed non-point source pesticide pollution (Miller et al., 2002). In such watersheds, the great advantage is that water is canalized through tile drains and drainage ditches which are easily identified and at which outlet it may be useful to construct wetland systems.
- Few studies assessed artificial wetlands efficiency receiving water from small watersheds where concentrations are high (Schulz, 2004).
- Many studies dealt with simulated and unrealistic conditions particularly concerning wetland influent pesticide concentrations sometimes higher than on-site recorded concentrations (Moore et al., 2000; Moore et al., 2001b; Moore et al., 2002; Runes et al., 2003; Sherrard et al., 2004). There is a lack of studies concerning wetland efficiency to decrease pesticide concentrations under real conditions.
- Few data were gathered for weakly sorbing pesticides, except the frequently monitored atrazine (Reichenberger et al., 2007) which use is forbidden in Europe.
- There is a lack of knowledge about pesticide removal processes (transfer and transformation) in wetland systems. In addition, internal hydraulics are rarely linked to pesticide dynamics (Kidmose et al., 2010).
- Besides wetland efficiency to reduce pesticide concentrations and loads, the portion of water coming out of a watershed and being treated through wetlands is almost never provided. However, if only a portion of contaminated water can be treated due to reduced wetland volume because of limited land-availability, this should be explicitly stated (Sherrard et al., 2004; Haarstad and Braskerud, 2005).
- Apart from researchers at the university of Mississippi (USA, Moore et al.), few attempts were made to provide design guidelines for constructed wetlands treating pesticide pollution.

## 7 Dissertation objectives and outline

This Ph.D program focused on the efficiency of an artificial wetland and a forest buffer at reducing pesticide pollution coming from a tile-drained agricultural catchment. The dissertation outline is described below and in Fig. 8.

Understanding and optimizing buffer zones functioning first requires a good knowledge of the way and timing pesticides are transferred through an agricultural watershed. This would help determining which flows are of most concern for pesticide transfer and should therefore be uppermost treated. Chapter I deals with this subject of first importance through the study of a tile-drained agricultural watershed hydrology and pesticide transfer dynamics located at Bray (Indre-et-Loire, France).

In addition, in order to be able to provide design guidelines, a good knowledge of the processes governing pesticide fate, and the way on-site full-scale buffer zones work under real conditions is needed. Several space and time scales would provide different levels of understanding.

First, laboratory experiments with radio-labelled molecules were carried out to assess retention (adsorption – desorption) and transformation (degradation, mineralization) processes (Chapter II) of selected pesticides. These small-scale experiments, conducted under controlled conditions, enabled expressing a maximal potential of pesticide processes under the selected conditions.

Second, increasing either space and / or time scales through on-site tracer experiments helped cross one more step in real systems' functioning and understanding of pesticide fate (Chapter III) under dynamic conditions. However, this intermediate scale remains limited to approach artificial wetland or forest buffer functioning under realistic conditions.

Consequently, two on-site systems, a forested buffer and an artificial wetland were implemented and monitored for three years (2007 – 2010). At this large space and time scales, systems' inlet ant outlet flow and pesticide data were collected thus providing an overall knowledge of their functioning (Chapter IV). At these scales, systems can be considered as "black boxes" whose internal processes can not be demonstrated, but efficiency under real conditions can be estimated.

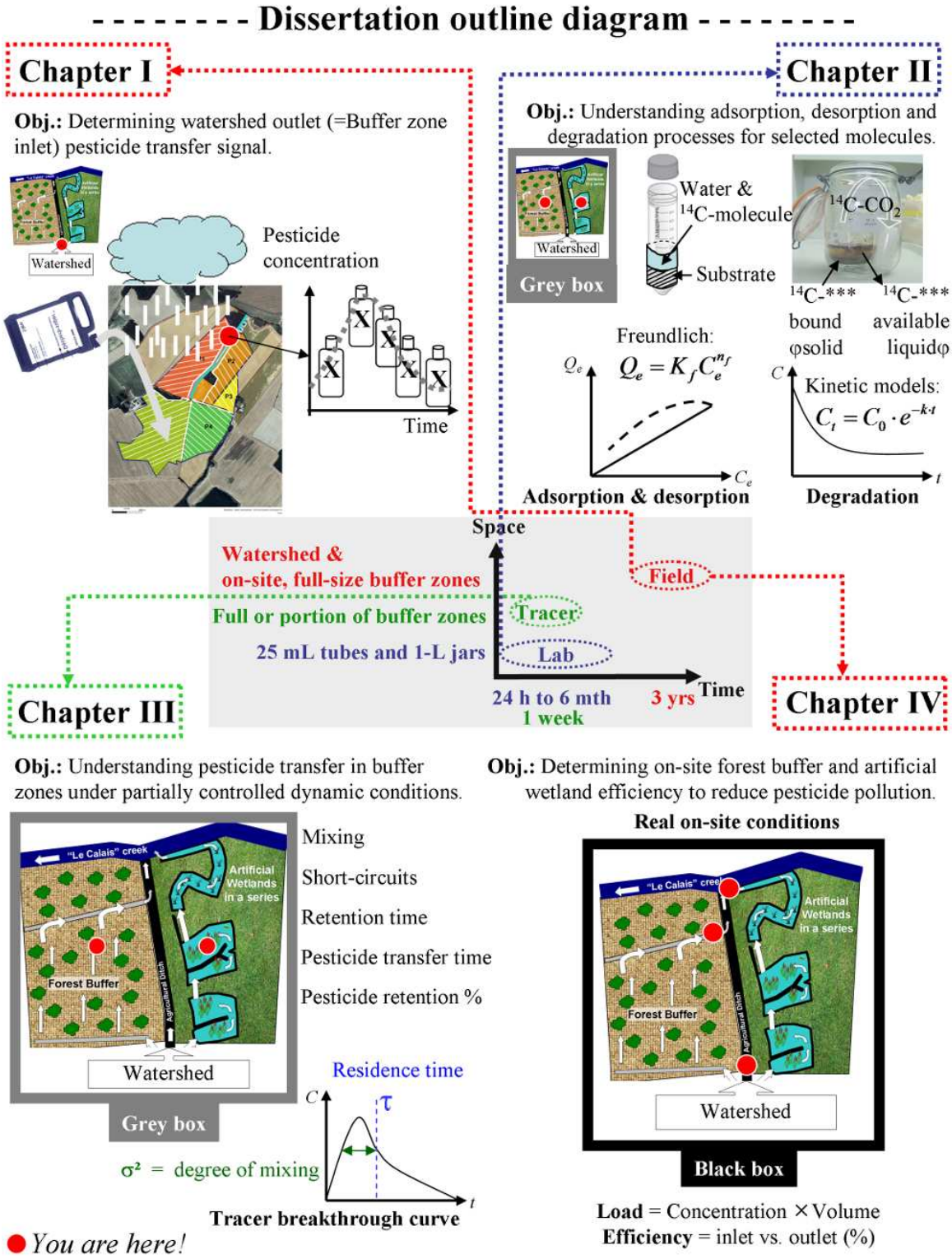
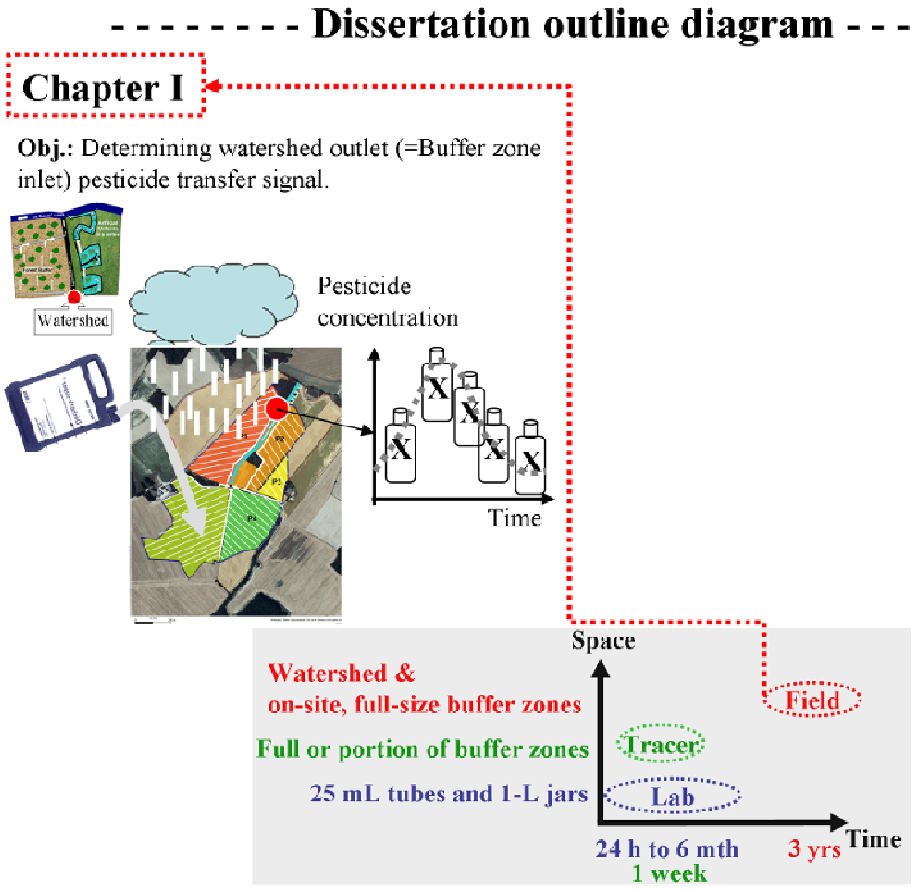



**Fig. 8: Dissertation outline diagram**

# CHAPTER I: NON-POINT SOURCE PESTICIDE POLLUTION IN THE TILE-DRAINED BRAY CATCHMENT, FRANCE




● *You are here!*

## 1 Introduction

To comply with current regulation objectives concerning water quality, like those presented in the European Water Framework Directive (European Union, 2000), actions are needed to reduce hydrosystem pesticide pollution. Among them, reducing pesticide applications, as requested through the French EcoPhyto program (French government, 2008), should be part of the solution. However, for the remaining pesticide loads reaching water bodies, complementary measures must be implemented. The latter could be achieved by buffer zones like artificial wetlands, grass or forest buffers, vegetated ditches or detention ponds (Reichenberger et al., 2007; Gregoire et al., 2008). Land availability is one of the most critical points to be addressed during the preliminary phase of buffer zone implementation. Farmers may accept to give up a reduced portion of their agricultural lands for such systems which would also imply reduced cropped area and associated economical benefits. Consequently, a good knowledge of pesticide transfer pathways from the agricultural field to receiving waters is needed to help decide where in the watershed buffer zones should be implemented and when they should receive polluted waters.

Pesticide input in the environment is due to human activities. For agricultural watersheds, the main input pathway consists of farmer pesticide applications even if atmospheric deposition via solid particles, rain and snow falls can partly contribute to pesticide input at the farm scale (Dubus et al., 2000).

Three major application methods referred to as spraying, incorporation into the soil and fumigation, lead to pesticide losses to the non-target environment. Indeed, only a portion of the applied product is taken up by plants and provides disease protection and/or weed elimination. When being applied, pesticide losses in the air typically range between a few percent to 20 – 30 % of the applied active substance, during application (mainly because of spray drift) and from a few percent to 50 – 60 % after application (by volatilization) and can sometimes reach up to 90 % (Van den Berg et al., 1999; Aubertot et al., 2005). Once on the soil, pesticide molecules undergo several transfer processes. Groundwater contamination is mainly due to pesticide leaching through infiltration; whereas, pesticide input pathways to surface water preferably come from surface runoff or tile drainage.

At the watershed scale, pesticide losses via surface runoff most frequently represent less than 1 % of the applied active substance rarely exceeding 10 % (Carter, 2000; Aubertot et al., 2005). The higher the soil water content, the higher the losses via surface runoff of the active substance and its metabolites. Moreover, because of soil surface erosion, molecules adsorbed onto the surface of soil constituents can also be transported to receiving surface waters. However, for flows presenting reduced suspended sediments concentrations, pesticide transport via surface runoff essentially affects soluble molecules; whereas, less mobile pesticides are less prone to be transferred (Aubertot et al., 2005).

On artificially drained watersheds, runoff is limited while tile drainage is the major pathway exporting excess water to surface waters (Kladivko et al., 2001; Augeard et al., 2005). Losses via subsurface drainage are generally less than 0.5 % of the applied pesticide dose but might reach 3 % and occasionally greater values (Ng et al., 1995; Garmouma et al., 1997; Chevreuil et al., 1999; Carter, 2000; Accinelli et al., 2003; Boivin et al., 2006). Most of previously published data were carried out at plot or small watershed outlets where exportations are usually greater than for larger-scale catchments. However, reviewing a wide range of studies, Kladivko et al. (2001) concluded that tile drainage concentrations and loads are up to one order of magnitude lower than those of surface runoff. This study also highlighted that even if subsurface drains represent an additional exportation pathway, the reduced rates of surface runoff losses were much more than the incremental rates of subsurface drainage losses. When crossing the soil from surface to subsurface drains,

pesticides can be involved in different retention or transformation processes. To sum up, it is clear that pesticide entries in the environment via surface runoff or subsurface drainage only represent a small amount of the applied active substance. On the other hand, it is important to note that the resulting concentrations and loads may be high enough for receiving surface waters to exhibit biologically relevant effects (Schulz, 2004).

Factors affecting pesticide transport to surface water via subsurface drainage are linked to soil, pesticide and agroclimatic characteristics (Brown and van Beinum, 2009). Pesticide molecules can be transported either in dissolved or adsorbed form on suspended solids, the former usually predominating (Aubertot et al., 2005). Soil composition accounts for pesticide movement. It is usually observed that, the higher the organic matter or clay content, the larger the retention of pesticides which reduces their transfer (Calvet et al., 2005a). It is obvious that pesticide retention and degradation characteristics are of importance for assessing their potential to be transferred by subsurface drainage or surface runoff. As for surface runoff generation, soil water content is a key parameter affecting water and solute transport to tile drains. Among laboratory experiments dealing with pesticide degradation on agricultural soils, some included time evolution of pesticide extractability by water (Boivin et al., 2004; Mamy et al., 2005; Alletto et al., 2006). Such studies can provide preliminary information about expected pesticide transfer loads under on-site field conditions. For instance, Boivin et al. (2004) demonstrated that bentazone extractability with water (0.01 M $CaCl_2$) can be important despite decreasing in time from 92–97 to 77–83 % during the first 7 days after its application for three different soils. It showed that bentazone may be easily transported by water flows particularly after its application. These results were subsequently well reproduced by bentazone concentration modelling at the outlet of a tile-drained watershed using HYDRUS-2D (Boivin et al., 2006).

When comparing dynamics of drainflows and the corresponding pesticide concentrations and loads, it appears that the highest concentrations occur during the first significant storm event following pesticide application (Garmouma et al., 1997; Kladivko et al., 2001; Novak et al., 2001; Jouzel, 2006). During a storm event, pesticide concentrations can vary over several orders of magnitudes (Schulz et al., 1998) with concentration peaks occurring generally just before drainflow peaks. A steep concentration decrease after pesticide concentration peak is usually observed while drainflow drops down (Kladivko et al., 2001). The next storm events usually present lower pesticide concentrations and loads. This is mainly due to the fact that the longer the pesticide or metabolite molecules remain in the soil, the more likely immobilization and degradation processes can occur, thus limiting the available quantity for transfer to natural surface water. This supports the fact that the first drainflow events after pesticide application are of most concern for pesticide pollution transfer (Schulz, 2001).

The objectives of this chapter were: (1) to characterize the hydrological functioning of the Bray artificially drained watershed, (2) to determine the doses and dates of pesticide applications, (3) to find the periods of most concern for pesticide transfer in order (4) to define where to implement buffer zones and which flows to preferentially treat.

## 2 Site description

The Bray experimental site is located 63 km southeast of the city of Tours, Indre-et-Loire, in the Centre region of France (longitude 47°3'50" N and latitude 1°17'4" E) (Fig. I-1). It is a 46-ha agricultural watershed with an average slope of 1.8 %, and a mean elevation of 137 m. The soil is hydromorphic and referred to as a Gleyic Luvisol (FAO) or Aqualf Alfisol (US Soil Taxonomy). It has a flint formation on a substrate with a clayey texture. Two geological strata dating from the upper Cretaceous period show on the surface in two distinct parts of the watershed (Alcaydé, 1990; BRGM, 2010), herein referred to as the upper and the

lower parts (Fig. I-2). In the upper part, the geological substrate dates from the Conacian to Campanian ages and is dominated by a clay-with-flint layer (green area, Fig. I-2b). This layer has a 20- to 30-m depth. Below is an aquifer located in a calcareous layer ("Tuffeau jaune"), dating from the late Turonian age. In the lower part of the watershed, and similarly below the buffer zones, the chalk layer is closer to the surface and is only covered by a thin (approximately 5 m) clay-with-flint layer. The clayey soil and the nature of first geology layer tend to indicate that slow infiltration can be expected. The aquifer is closer to the surface (somewhere below 5 m) below the experimental buffer zones and may be potentially at risk.

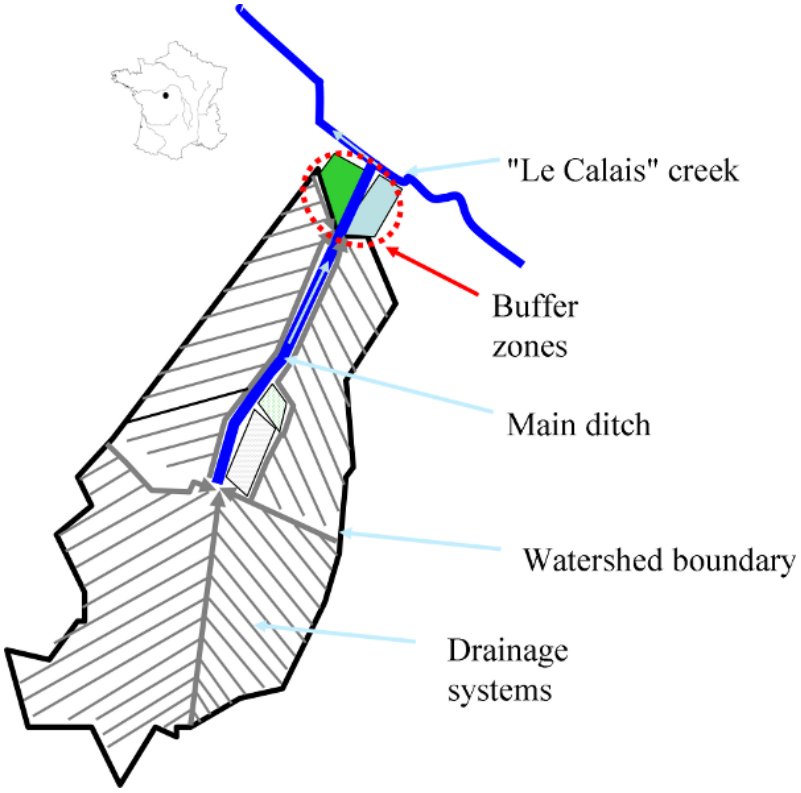



**Fig. I-1: Bray watershed (46 ha) diagram indicating buffer zones locations (green: forest buffer; blue: artificial wetland).**

Not surprisingly, given the previous geological information, the whole catchment has been artificially drained since the 1960s by 90-cm-deep and 10-m-spaced subsurface drains lying on a clay accumulation layer (design project discharge is 1.39 L/s/ha). Overland runoff production is therefore limited. Rainfall events generate tile drainage waters that are exported to a main collecting ditch prior to reaching a natural creek called Le Calais, a tributary of the Indre River. Long-term average annual rainfall, potential evapotranspiration, and temperature are approximately 778 ± 143 mm, 857 ± 66 mm, and 11.5 °C, respectively. Rainfall is almost evenly distributed throughout the year with monthly rainfalls ranging from 51 (June) to 79 (November) mm. On the Bray watershed, one farmer and his son mainly grow winter wheat (*Triticum aestivum* L.), rape (*Brassica napus* L.), and winter barley (*Hordeum vulgare* L.) (Table I-1). The agricultural watershed is divided into five plots (Fig. I-3) whose surface areas range from 3 to 16 ha (Table I-1).

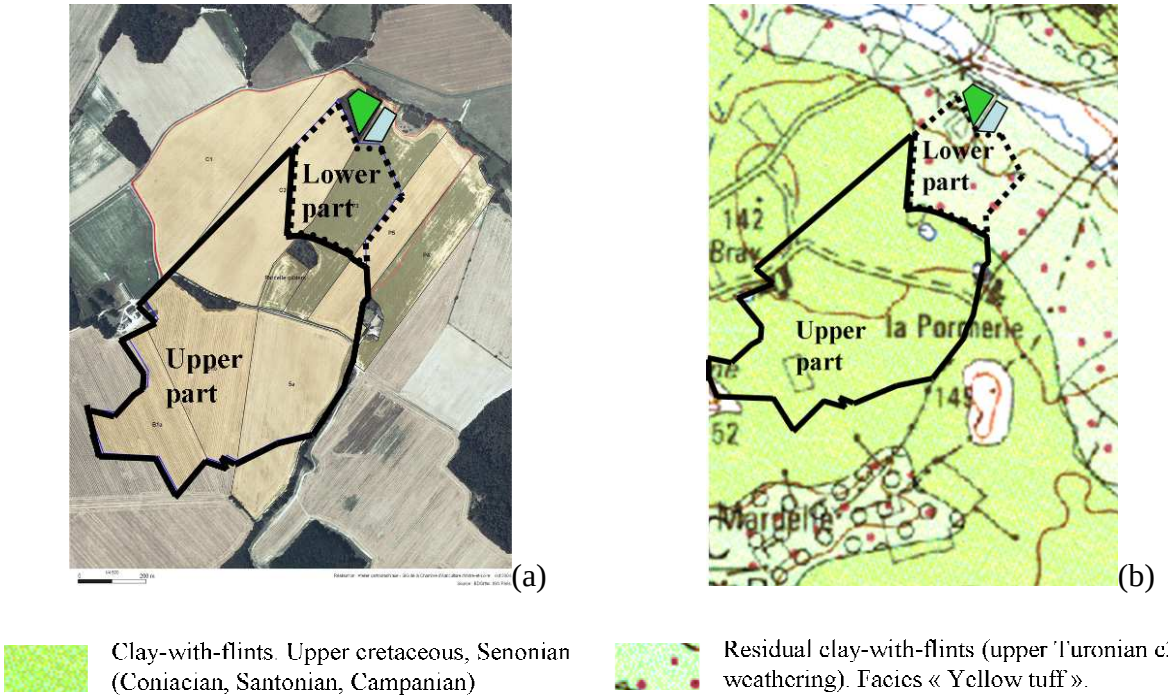



**Fig. I-2: Bray watershed (46 ha). (a) Satellite view and watershed limitation (solid and dotted lines) (IGN map); (b) Geology map corresponding to the Bray watershed.**

| Plot | P1 | P2 | P3 | P4 | P5 |
|---|---|---|---|---|---|
| **Surface area (ha)** | 14 | 6 | 3 | 7 | 16 |
| **2002-03** | rapeseed | wheat | sunflower | wheat | winter barley |
| **2003-04** | wheat | sunflower | wheat | rapeseed | rapeseed |
| **2004-05** | winter barley | wheat | sunflower | wheat | wheat |
| **2005-06** | rapeseed | millet | wheat | rapeseed | winter barley |
| **2006-07** | wheat | N/A | sunflower | wheat | rapeseed |
| **2007-08** | winter barley | sunflower, corn, sorghum | wheat | rapeseed | wheat |
| **2008-09** | rapeseed | millet, corn, sunflower | sunflower | wheat | winter barley |
| **2009-10** | wheat | millet, corn, sunflower | wheat | rapeseed | rapeseed |

**Table I-1: Crop rotation on the Bray catchment (2002-2010). N/A stands for "not available".**

## 3 Materials and methods

### *3.1 Pesticide inputs*

All data concerning the watershed land-use and pesticide applications were extracted from the farmer records and are presented in Appendix III.

### *3.2 Monitoring equipment*

The outlet of the watershed also corresponds to the inlet of an artificial wetland (on the right hand of the main ditch) and a forest buffer (on the left hand of the main ditch) (Fig. I-3), further described (Chapter III). The volumes of water coming out of the Bray catchment can either enter one or both of the buffer zones, or go straight through the main ditch, down to "Le Calais" natural creek. Three flow paths are therefore possible for the watershed outlet flows according to buffer zones openings and closings (Fig. I-4). Consequently, total flow

measurement coming out of the watershed was obtained thanks to different equipments. Two electromagnetic flow meters (MAG 8000 SIEMENS) were set up at the inlets of both mitigation systems. They were placed inside PVC pipes whose diameters were limited (Fig. I-4). The artificial wetland inlet pipe diameter was 200 mm limiting the flow rate to 35 L/s approximately (Fig. I-4).


**Fig. I-3: Plot distribution on the Bray catchment. The part of P2 in orange without hatchings was usually not cultivated.**

In the forest buffer, the inlet diameter was 200 mm until 18 February 2009. At this date, forest inlet was controlled to get a 0.3 L/s flow rate for a tracer experiment (see Chapter III) until 05 March 2009. Because of previous overflow observations at the forest buffer outlet, forest inlet diameter was reduced to 100 mm (maximal flow rate of approximately 9 L/s) after 05 March 2009 (Fig. I-4). However, the forest buffer has been closed on this date, consequently, inlet section reduction to 100 mm really started to influence flow rate from next hydrologic year (2009 – 2010). With these flow meters, measurement is only possible for full sections of the pipes. Discharges were recorded from September 2007 to June 2010. In addition, water level measurement devices (SE-200 OTT and Druck PDCR1830 sensors) were installed in 90° V – notch sections situated in the main ditch. Discharge gaugings were carried out to relate water levels to flow rates at these two measurement stations by means of rating curves. A tipping bucket rain gauge provided rainfall data on site. On-site rainfall data have been recorded since 2 April 2007. Beforehand and for periods of missing data, daily precipitation data were obtained from the Beaumont-Village meteorological station located 15 km away from the Bray experimental site. Continuously from 2007, flow-weighted composite samples were taken at the watershed outlet by means of an ISCO 3700 automatic sampler. The farmer's son was in charge of collecting these samples approximately every week. Depending on the flow passing through the main agricultural ditch, each sample concentration was therefore an average of the water coming out of the watershed. Sub-samples (100 mL) composing these weekly collected flow-weighted composite samples were programmed to be

taken approximately every 10 $m^3$. Occasionally, after selected pesticide applications, additional automatic samplers associated with 24 flasks were set up to take time-dependent samples every 2 to 12 hours, over short periods of time (1 to 3 weeks) (Fig. I-5).

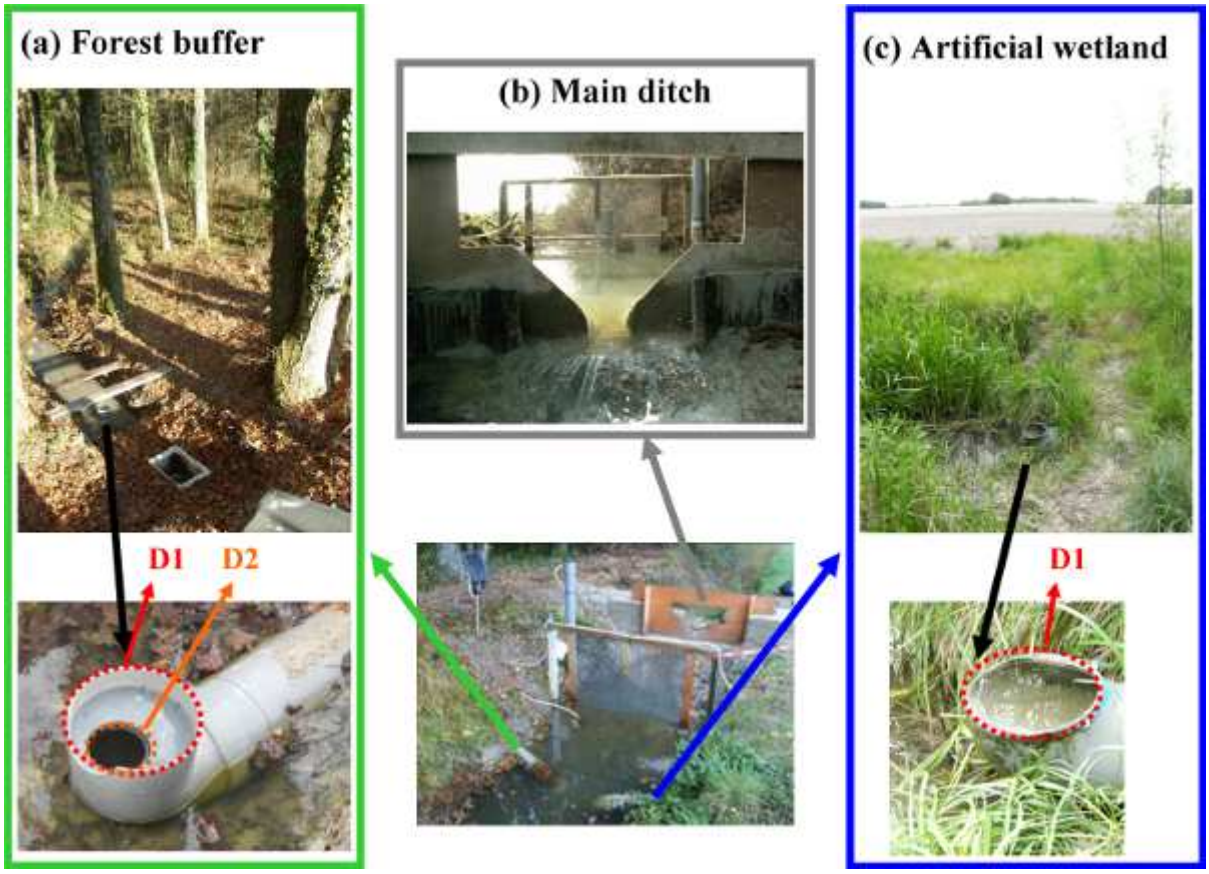



**Fig. I-4: (a) The three possible routes for watershed outlet flows: (a) forest buffer inlet, (b) agricultural main ditch and (c) artificial wetland inlet. Forest buffer and artificial wetland inlet PVC pipes are zoomed in. Forest PVC pipe inlet diameter was D1 = 200 mm up to 18 February 2009 when it was then reduced to D2 = 100 mm. Artificial wetland PVC pipe inlet diameter was 200 mm.**

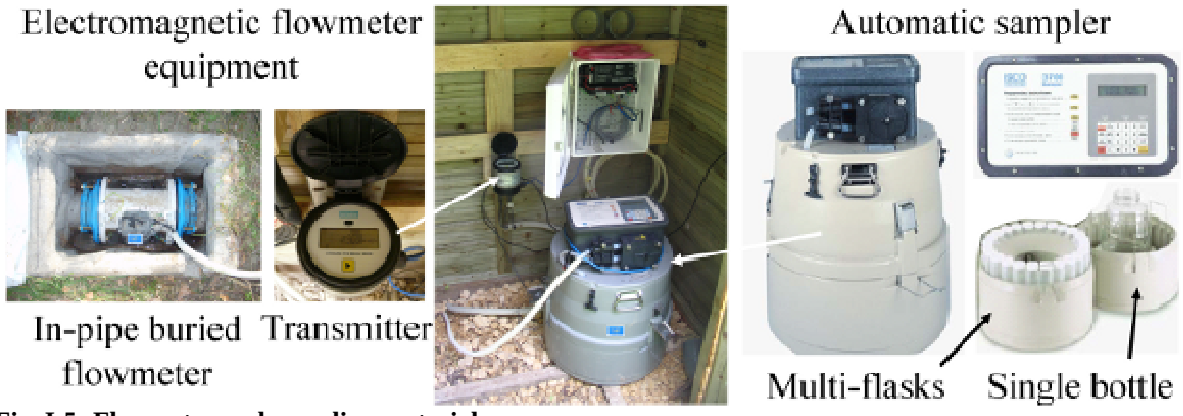



**Fig. I-5: Flowmeter and sampling material**

*3.3 Data analyzes*

Discharge values were recorded every 15 min. Peaks were defined as the maximum values of flow rates exceeding 5 L/s for each individual flood. Probability and statistical analyses were performed using both Microsoft Excel and R softwares (R Development Core Team, 2005). Descriptive statistics including mean, median, and standard deviation were

calculated. In addition, normal probability plots and Shapiro-and-Wilk tests ($\alpha$=0.05) were computed to test data normality. Flow rates were log-transformed to comply with a normal distribution. Probability of exceedance graphs were drawn for discharge peak analyzes. Data were first rearranged from the largest to the smallest value. Probability plotting positions usually serve as estimates of the probability of exceedance of the observed data (Guo, 1990). The proposed plotting position ($p_i$) developed by Blom (1958) is often recommended for normal distribution (Looney and Gulledge, 1985a; b):

$$p_i = \frac{i - 0.375}{N + 0.25} \quad \text{(Eq. 3)}$$

where i is the rank of the ordered sample data and N is the total number of observations.

The return period (T, year) for independent catchment outlet peak flow rates (Q, L/s or L/s/ha) exceeding a given threshold (5 L/s) may be estimated using the renewal model, a combinaison of an exponential and Poisson laws (Michel, 1991).

$$T = \exp(\frac{Q - b}{a}) \quad \text{(Eq. 4)}$$

Coefficients a and b were estimated as follows:

$$a = (1 + \frac{1}{k}) \cdot (m - Q_k) \quad \text{(Eq. 5)}$$

and

$$b = Q_k + a \cdot (\ln\frac{k}{n} - \frac{1}{k}) \quad \text{(Eq. 6)}$$

where k was the total number of recorded peak discharges (k=73), n was the number of sampled years (n=3) and m was the discharge average of the k peaks.

The volumes of watershed outlet tile drainage and rain were calculated. Rainfall restitution coefficients were estimated by dividing the total cumulated subsurface tile drainage volumes by the corresponding total rainfall volume (expressed as a percentage). This was determined graphically for each period from changes in the slopes in double-cumulated graphs as shown in Zimmer (1988).

*3.4 Sample analyzes*

Filtered (PET 20/15 MS Macherey-Nagel, VWR) sub-samples (17 mL) of water were analyzed after Solid-Phase Micro-Extraction (SPME) and Gas-Chromatography coupled to a Mass-Spectrometer (GCMS) (Trace DSQ, Thermo-Fisher Scientific). Details on extraction and chromatographic procedure, the whole method development and validation, as well as uncertainties calculations are presented in Passeport et al. (2010a) and reported in Appendix II. This analytical method enabled the simultaneous determination of 16 pesticides in water whose main characteristics are presented in Table I-2. Pesticide concentrations are therefore presented as their measured value ± the corresponding expanded uncertainty due to the analytical method. Suspended matters (MES) were determined after filtration through GF/F (45 µm) filters by means of a void pump. Nitrate ($NO_3^-$) concentrations were measured with ionic chromatography (DIONEX DX120) on filtered samples.

*3.5 Pesticide data analyzes*

Watershed outlet pesticide loads were estimated by multiplying the flow-weighted composite sample concentrations by the corresponding volume of drainage water exiting the watershed during the sampling period (approximately one week). Missing concentrations were substituted by the averages of the previous and next concentrations. In addition, data below the limit of quantification (LQ) were substituted by LQ divided by 5. The load of each pesticide was then summed over specific periods of interest. These data were compared to the corresponding annual application doses. Box-plots and histograms were plotted for watershed

outlet concentrations and normal and log-normal distributions were verified through Shapiro-and-Wilk tests ($\alpha$=0.05) with the R software.

| Pesticides | Log $K_{o/w}$ [(a)] | $K_{oc}$ [(b)] | $DT_{50,field}$ [(c)] | $DT_{50,sed}$ [(c)] | $DT_{50,water}$ [(c)] | GUS[(d)] | Henry Constant (25 °C) | Water solubility (20 °C) | Chemical group |
|---|---|---|---|---|---|---|---|---|---|
| | - | $mL.g^{-1}$ | d | d | d | - | $Pa.m^3.mol^{-1}$ | $mg.L^{-1}$ | |
| **Herbicides** | | | | | | | | | |
| aclonifen | 4.37 | 7126 | 117 | 14.3 | 4.2 | 0.3 | $3.03\times10^{-3}$ | 1.4 | Nitrophenyl ethers |
| atrazine | 2.7 | 100 | 29 | 80 | N/A | 3.75 | $1.50\times10^{-4}$ | 35 | Triazines |
| chlorotoluron | 2.5 | 205 | 34 | 352 | 42 | 2.79 | $1.44\times10^{-5}$ | 74 | Ureas |
| mefenpyrdiethyl | 3.83 | 634 | 17.5[(a)] | 135 | 80 | 1.49 | $2.55\times10^{-4}$ | 20 | *Pesticide safener* |
| metazachlor | 2.49 | 134 | 6.8 | 20.6 | 216 | 1.75 | $5.90\times10^{-5}$ | 450 | Chloroacetamides |
| isoproturon | 2.5 | 122 | 23 | 149 | 40 | 2.07 | $1.46\times10^{-5}$ | 70.2 | Ureas |
| napropamide | 3.3 | 885 | 72 | 316 | 28 | 1.94 | $8.10\times10^{-5}$ | 74 | Alkanamides |
| S-metolachlor | 3.4 | 200 | 21 | 365 | 88 | 3.32 | $2.40\times10^{-3}$ | 530 | Chloroacetamides |
| ethofumesate | 2.7 | 147 | 56 | 530 | 20 | 3.38 | $6.8\times10^{-4}$ | 50 | Benzofurans |
| prosulfocarbe | 4.48 | 1693 | 9.8 | 214 | 1.05 | 1.15 | $1.52\times10^{-2}$ | 13.2 | Thiocarbamates |
| diflufenican | 4.2 | 3186 | 315 | 175 | N/A | 1.36 | $1.18\times10^{-2}$ | 0.05 | Carboxamides |
| **Fungicides** | | | | | | | | | |
| chlorothalonil | 2.94 | 850 | 44 | 0.1 | 0.1 | 1.44 | $2.5\times10^{-2}$ | 0.81 | Chloronitriles |
| epoxiconazole | 3.3 | 1073 | 120 | 119.8 | 65.8 | 2.47 | $4.71\times10^{-4}$ | 7.1 | Triazoles |
| fenpropidine | 2.6 | 3808 | 49.2 | 34 | 1.8 | 0.82 | 10.7 | 530 | Morpholines |
| tebuconazole | 3.7 | 769 | 55.8 | 365 | 42.6 | 2 | $1.0\times10^{-5}$ | 36 | Triazoles |
| cyproconazole | 3.09 | 309 | 191 | 300 | 300 | 3.25 | $5.0\times10^{-5}$ | 93 | Triazoles |

**Table I-2: Main characteristics for the 16 pesticides belonging to the SPME – GC/MS analytical method (Passeport et al., 2010a). [(a)]Log $K_{o/w}$: octanol/water partition coefficient; [(b)]$K_{oc}$: sorption coefficient normalized to the organic content; [(c)]$DT_{50field}$, $DT_{50sed}$ and $DT_{50water}$: half-life for field soil, and in sediment and water phases from laboratory water/sediment studies; [(d)]GUS: Groundwater ubiquity score, leaching coefficient calculated by $[4-\log_{10}(K_{oc})].\log_{10}(DT_{50})$. All data were obtained from FOOTPRINT (2010).**

Toxic units (TU) were calculated for each molecule j flow-weighted composite or time-dependent sample concentration ($C_j$):

$$TU_{D.magna} = \log\left(\frac{C_j}{LC_{50\,j}}\right) \quad \text{(Eq. 7)}$$

Their determinations were based on the acute (48-h) letal-dose for 50 % of initial population ($LC_{50j}$) of *D. magna* (FOOTPRINT, 2010) to estimate possible impact of pesticides to wetland biota or the receiving stream. However, it is clear that further dilution of concentrations was expected once the buffer zones outlet flows enter the stream. TU ranging from -2 to -3 were considered "low", whereas those exceeding -1 were considered "high" TU (Liess and von der Ohe, 2005).

## 4 Results and discussion

### *4.1 Pesticide applications*

#### 4.1.1 Applied pesticides

From data obtained over an 8-year period (2002 – 2010), major herbicide application periods on the Bray catchment mainly corresponded to fall (August – November) and spring (April – May) and accounted for 85.3 % of total applied pesticides (Table I-3). Fungicide applications (12.1 % of applied pesticides, on average) occurred in April and May (Fig. I-6). There were occasionally other herbicide applications in late winter, early and late spring (Fig. I-6). These applications were related to the crops grown and differ from one plot to another (Appendix III). For instance, isoproturon is a mobile herbicide usually applied from October to December on winter wheat and barley crops, similarly to diflufenican and prosulfocarb.

| Year | Herbicides | | | Fungicides | | | Insecticides | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | kg | % | Nb mol | kg | % | Nb mol | kg | % | Nb mol | kg | Nb mol |
| 2002-03 | 133.7 | 81.9 | 16 | 29.4 | 18.0 | 9 | 0.1 | 0.1 | 1 | 163.2 | 26 |
| 2003-04 | 123.7 | 93.3 | 15 | 5.7 | 4.3 | 4 | 3.1 | 2.4 | 3 | 132.5 | 22 |
| 2004-05 | 57.8 | 74.2 | 14 | 20.1 | 25.8 | 7 | 0.0 | 0.0 | 0 | 77.9 | 21 |
| 2005-06 | 97.2 | 83.5 | 16 | 19.1 | 16.4 | 5 | 0.1 | 0.1 | 1 | 116.4 | 22 |
| 2006-07 | 42.8 | 78.2 | 11 | 3.3 | 6.0 | 2 | 8.6 | 15.8 | 2 | 54.7 | 15 |
| 2007-08 | 82.2 | 88.5 | 21 | 10.3 | 11.1 | 7 | 0.3 | 0.4 | 1 | 92.9 | 29 |
| 2008-09 | 96.4 | 91.8 | 21 | 8.3 | 8.0 | 6 | 0.3 | 0.3 | 2 | 105.0 | 29 |
| 2009-10 | 117.4 | 91.1 | 20 | 9.6 | 7.5 | 7 | 1.8 | 1.4 | 2 | 128.8 | 29 |
| **Average** | **93.9** | **85.3** | **17** | **13.2** | **12.1** | **6** | **1.8** | **2.5** | **2** | **108.9** | **24** |

**Table I-3: Total pesticide inputs on the Bray catchment (46 ha) per cultural year (August to July of the following year) since 2002. % are the percentages of applied masses per pesticide type (herbicides, fungicides, insecticides) compared to the total number of different applied molecules.**

Winter wheat also received fungicide applications (epoxiconazole) in spring. Winter barley and wheat received similar pesticide applications and grew during almost simultaneous periods. Rapeseed was sown earlier than wheat and barley, approximately in August, and therefore received earlier herbicide application of metazachlor and napropamide. Finally, metazachlor and aclonifen herbicides were spread in spring on sunflower crops. The number of different molecules, as the molecules themselves, that were applied on this catchment did not significantly change during the period of study (from 2007 to 2010) (Table I-3). There are uncertainties on pesticide records in the years preceding those of this study. Data were missing for pesticide application dates, for instance in 2005-06 (Appendix III). Insecticides, molluscicides were applied on the catchment but with a lower frequency and applied doses than those of herbicides and fungicides. As some of pesticides applied before 2007 may still be present in the Bray watershed soil, for not having being degraded or transferred yet, it was chosen to provide this information in Appendix III.

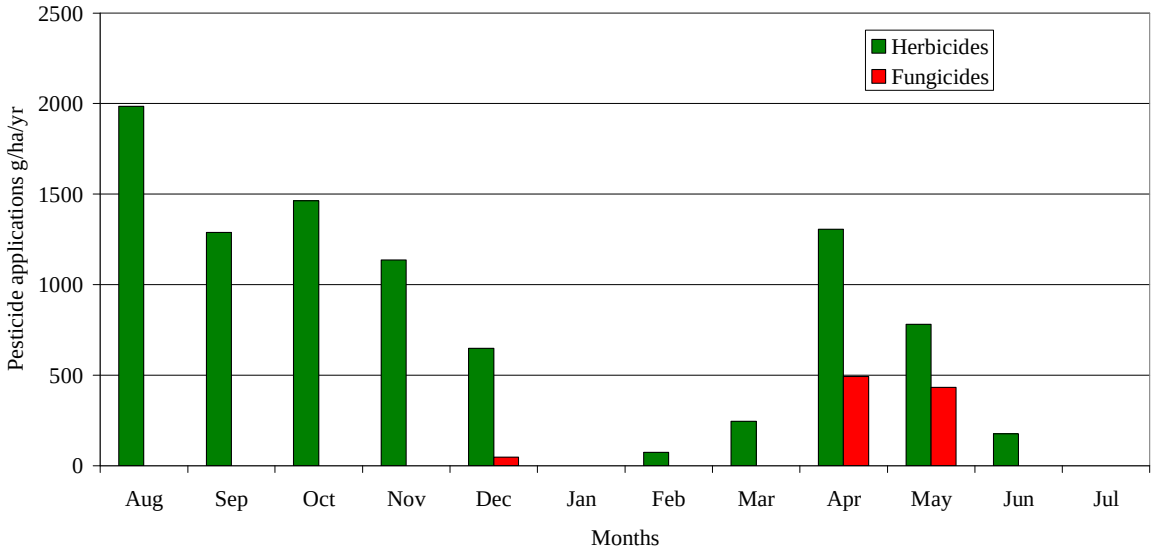



**Fig. I-6: Average (on the 8-year period) herbicides and fungicides applied doses at Bray.**


**Fig. I-7: Calendar overlapping crop cultural cycle (red lines), pesticide application (green lines, for molecules belonging to the analytical method) and approximate intense drainage season (blue columns) at Bray.**

### 4.1.2 Molecules belonging to the analytical method

Among the 16 pesticide molecules that could be analyzed by the SPME-GCMS analytical method (Appendix IV) and Passeport et al. (2010a), 9 were applied by the farmer during the monitoring period (2007-2010): chlorotoluron, napropamide, diflufenican, isoproturon, epoxiconazole, aclonifen, mefenpyrdiethyl, metazachlor, prosulfocarb. In addition, apart from prosulfocarb, the previous molecules, as well as cyproconazole, were also applied from 2002 to 2007 (Appendix III). Compared to the total applied mass of herbicides and fungicides employed by the farmer, 25 (2007 – 2008), 41 (2008 – 2009) and 52 (2009 – 2010) % of the applied pesticides belonged to the sixteen molecules analyzed by the SPME-GCMS analytical method. Consequently, despite the fact that it encompassed only a small

portion of the total number of molecules (approximately 20 %), a non-negligible portion of pesticide masses actually spread onto the watershed was evaluated.

*4.2 Hydraulic functioning of the sub-surface artificially drained Bray catchment*

Because of subsurface drainage flow seasonality, data were gathered from 1 October to 30 September of the following year. For instance, data collected from 1 October 2007 to 30 September 2008 was called the 2007 – 2008 hydrologic year. Inter- and intra-annual variability is explained by tile drainage flows depending strongly on climatic factors (rain and ETP) and the watershed soil's saturation state.

In 2007 – 2008, total rainfall was 777 mm and total potential evapotranspiration (PET) was 785 mm (Fig. I-8). Overall, 2007 – 2008 can be considered an average year but was characterized by a wet spring. Watershed outlet flow rate values (15-min time-step), mainly consisting of subsurface pipe drainage flows, reached up to 537.0 L/s in 2007 – 2008 (Fig. I-9) after a 54 mm rainfall event (27 May 2008) which corresponded to a 10-year return period. In 2008 – 2009, annual rainfall and PET were 595 and 835 mm, respectively which indicates that 2008 – 2009 could be considered as a dry year (2008 – 2009 annual rainfall < [long-term annual rainfall – standard deviation]). Maximal watershed outlet peak flow rate was 93.8 L/s (occurring on 24 January 2009). In 2009 – 2010, rainfall and PET were 677 and 857 mm, respectively, up to Sept 19$^{th}$ 2010. This PET is in the average of long-term values but 2009 – 2010 rainfall depth was fairly lower for that period of time than long-term records. Maximal outlet peak discharge was 138.7 L/s (on 6 February 2010).

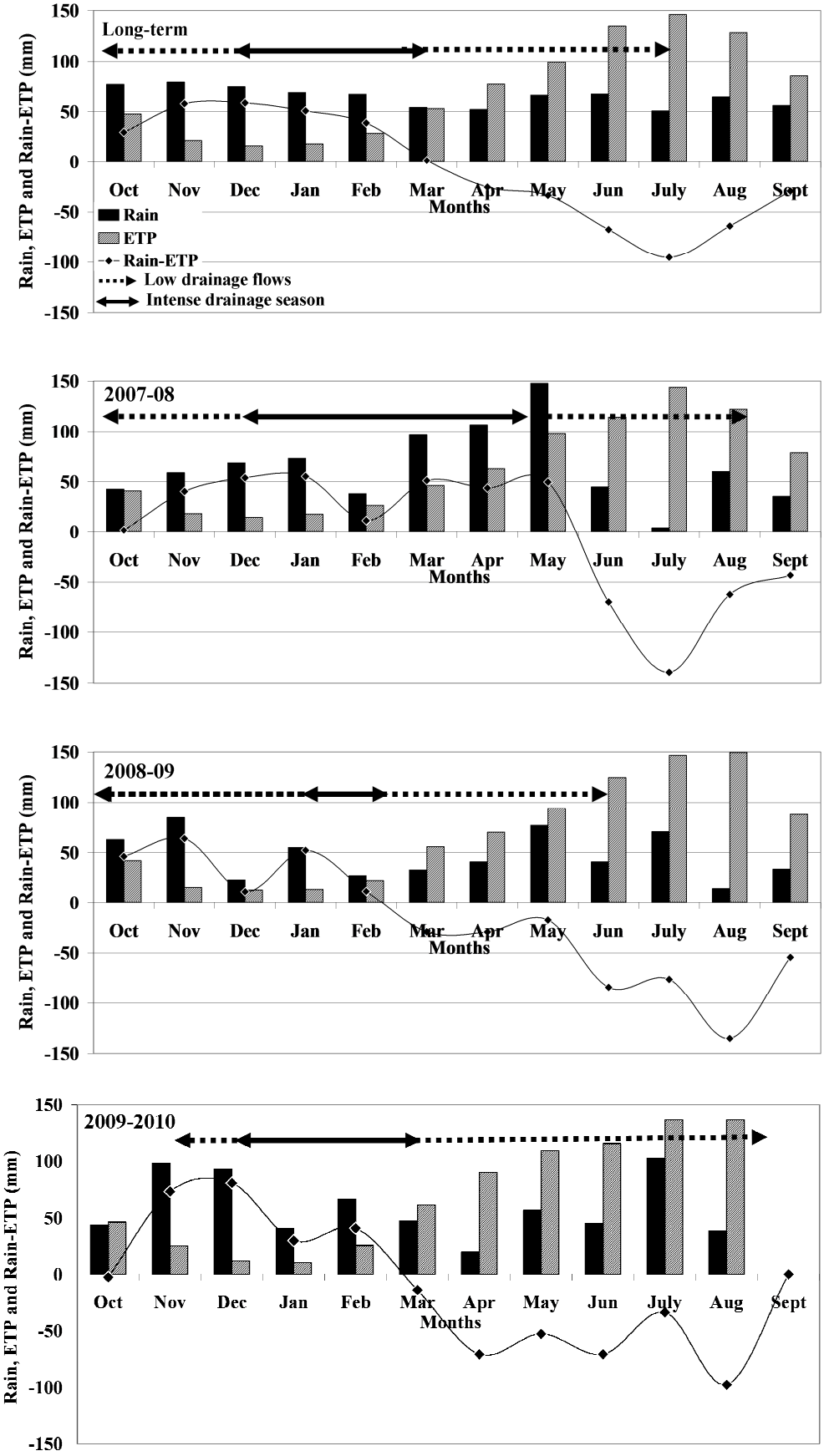
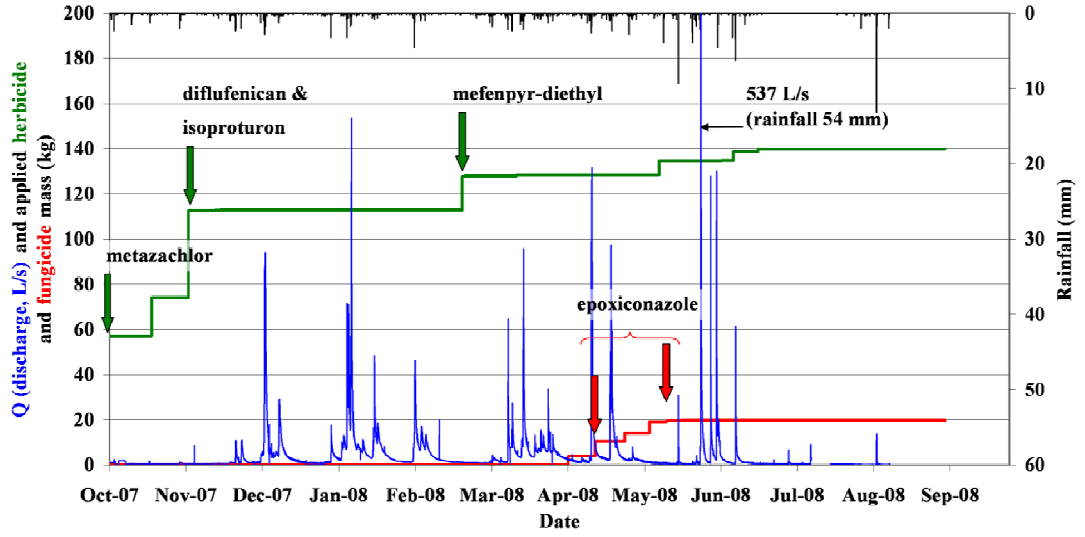
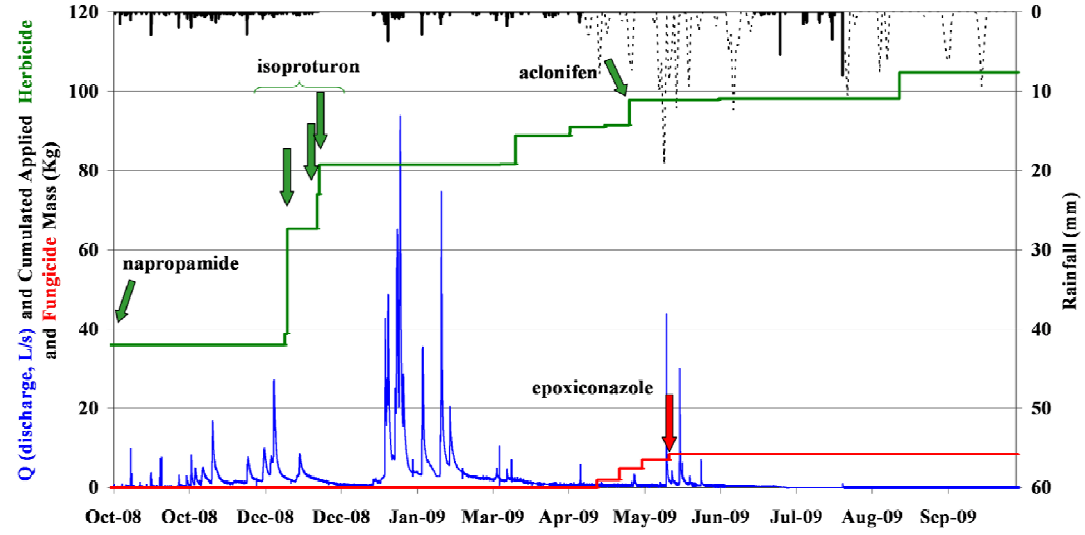


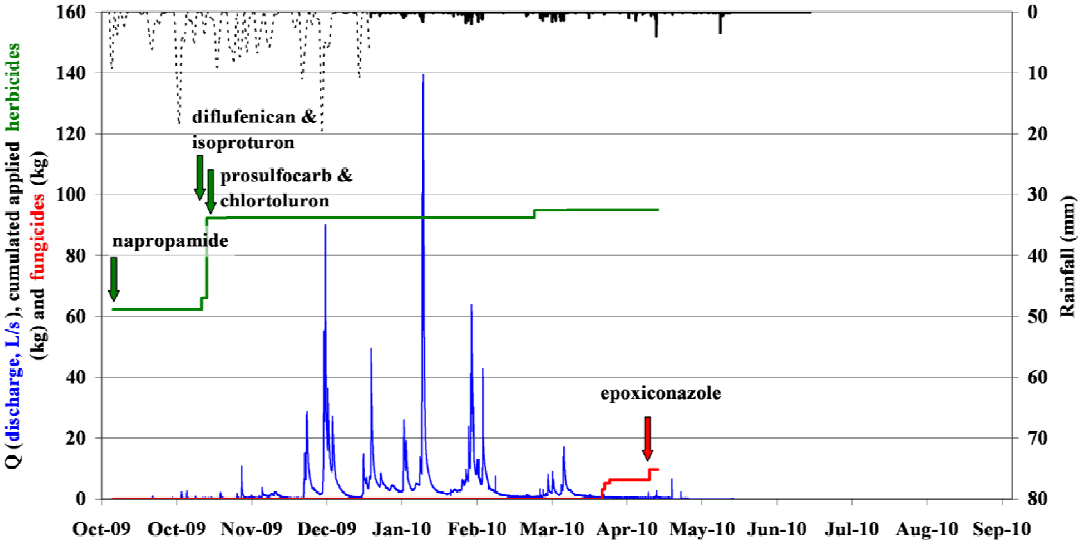

**Fig. I-8: Long-term and the three-year monitoring period rainfall, potential evapotranspiration (PET) and indication of periods of flows and intense drainage season at the catchment outlet.**




(a)




(b)




(c)

**Fig. I-9: Bray watershed outlet flow rates, rainfall and pesticide applications. Applications of molecules that can be analyzed by the analytical method are indicated by the arrows. For periods of missing Bray 15-min rainfall data (full black lines), Beaumont Météo-France daily data (dotted black lines) were substituted.**

The statistical analysis of the whole (2007–2010) set of watershed outlet independent peak flow rates (n = 54) showed that on average, 44 % and 90 % of the watershed outlet peak flow rates exceeded 35 L/s (0.76 L/s/ha) and 9 L/s (0.20 L/s/ha), respectively (Fig. I-10). The 35 L/s and 9 L/s thresholds are of particular interest in the present study as they correspond to the maximal discharges that could pass through the buffer zones' inlet PVC pipes (200 or 100 mm diameter, respectively). 35 L/s and 9 L/s flow rate return periods were 37 and 22 days, respectively. Whereas flood peaks of 90 L/s for instance, had a return period of approximately 3.8 months (116 days).

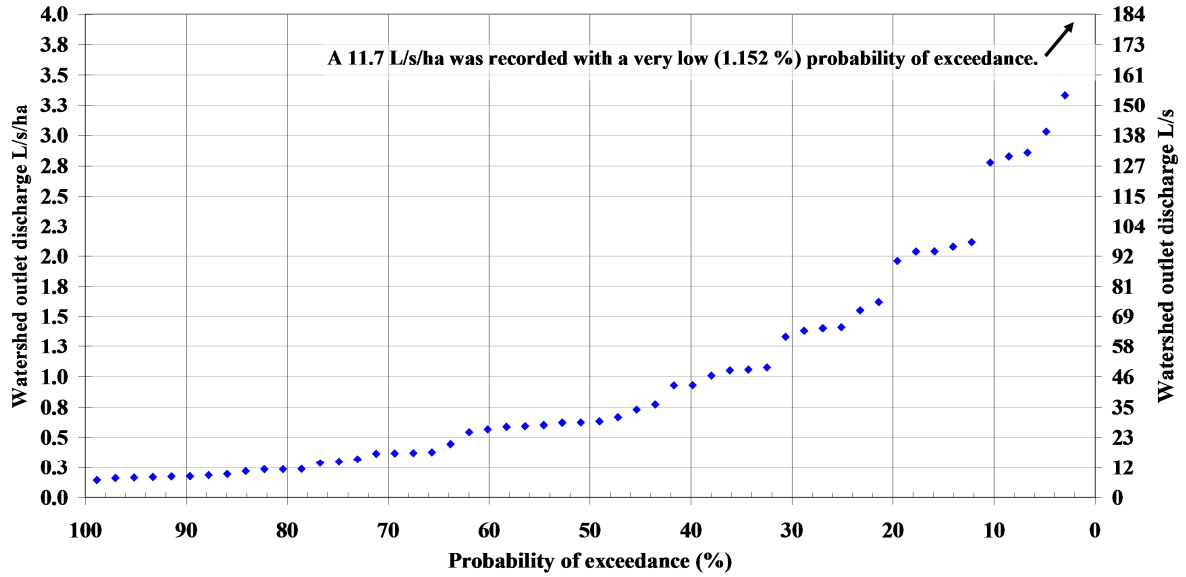



**Fig. I-10: Probability of exceedance graph for Bray watershed outlet flow rates given in L/s/ha (left hand Y-axis) and L/s (right hand Y-axis).**

The same study was done considering only peak flow rates occurring during the six major pesticide applications months (n = 21): September to November and March to May (Fig. I-6). Despite presenting high application rates, August was not included for not being associated with flow rates as drainage was not functioning yet in any of the three year of records (Fig. I-9). 36 % of watershed outlet peak flow rates during these months were larger than 35 L/s (Fig. I-11) whose return period, on these specific months, was approximately 3 months (88 days).

The total annual pipe drainage volume varied from 62 203 (i.e. 135 mm) to 109 192 $m^3$ (i.e. 237 mm) for 2009 – 2010 and 2007 – 2008, respectively (Table I-4). Three distinct hydrologic periods are generally observed for temperate climate areas associated with artificially drained watersheds in North-western Europe, as for the Bray catchment (Table I-4, Fig. I-12). The first rainfall events of the hydrologic period do not trigger large pipe drainage flow rates and volumes at the watershed outlet but only first sporadic outflows. On the Bray catchment, this occurred in October – December and was associated with 6 to 20% rainfall restitution coefficients (Fig. I-12). In 2009 – 2010, the first drainage flow rates started later (mid-November 2009) than in the first two years of the monitoring period. Once the soil reached its water storage capacity, subsequent to 142 to 218 mm of rainfall, pipe drainage discharge response to precipitation was faster. This is the intense drainage season. In 2007 – 2008, this period (from 7 December 2007 to 28 April 2008) was unusually characterized by two distinct periods presenting 72% (winter) and 43% (early spring) restitution coefficients (Fig. I-12). The other two years were characterized by more commonly observed restitution coefficients of 75 (2007 – 2008) and 89 (2009 – 2010) % during the intense drainage seasons (Table I-4 and Fig. I-12). More frequent and larger rainfalls occurred in spring 2008 compared to long-term values (Fig. I-9). Finally, as rainfall frequency decreased and

evapotranspiration increased, tile drainage discharges dropped until the next hydrologic period started with episodic flows. This hydrologic functioning is typical for North-western Europe. For example, similar trends were observed on other artificially drained watersheds in Germany (Tiemeyer et al., 2006).

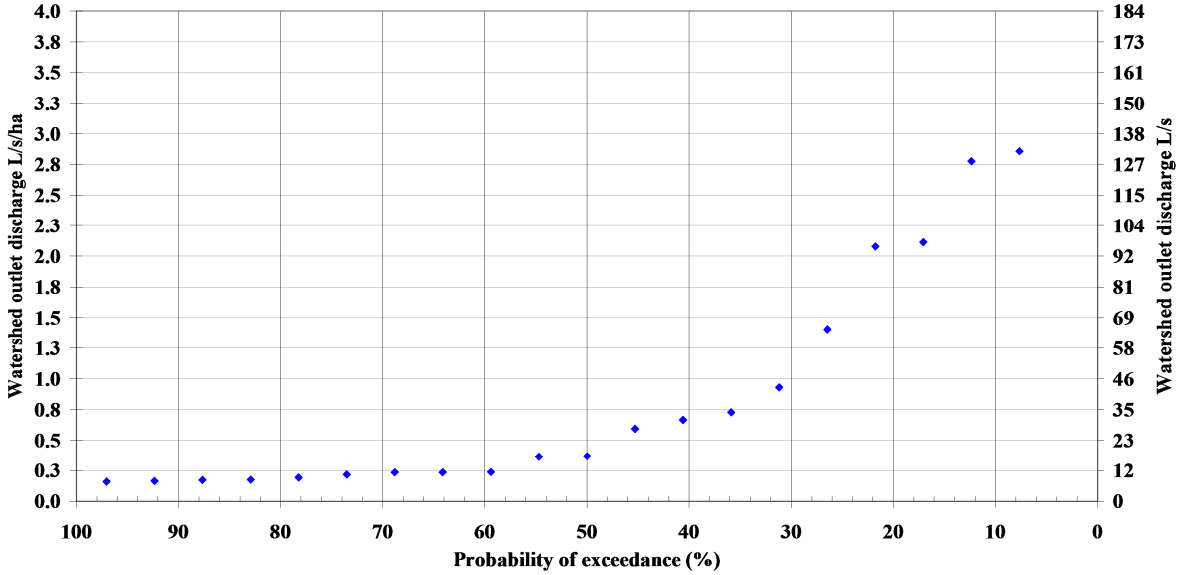



**Fig. I-11: Probability of exceedance graph for Bray watershed outlet flow rates given in L/s/ha (left hand Y-axis) and L/s (right hand Y-axis) recorded during the major pesticide application months (sept – nov and mar – may).**

| | 2007 – 2008 | 2008 – 2009 | 2009 – 2010 |
|---|---|---|---|
| **Drainage initiation** | | | |
| Period | 1 Oct. 2007 – 07 Dec. 2007 | 30 Oct. 2008 – 02 Dec. 2008 | 18 Nov. 2009 – 28 Dec. 2009 |
| Rain depth (mm) | 11 | 69 | 101 |
| Restitution Coefficient (%) | 20 | 10 | 6 |
| **Intense Drainage Season** | | | |
| Period | 07 Dec. 2007 – 28 Apr. 2008 | 02 Dec. 2008 – 26 Mar. 2009 | 28 Dec. 2009 – 18 Mar. 2010 |
| Rain depth for IDS initiation (mm) | 140 | 170 | 218 |
| Restitution Coefficient (%) | 55 (72 and 43) | 75 | 89 |
| **End of drainage** | | | |
| Period | 29 Apr. 2008 – 30 Sept. 2008 | 27 Mar. 2009 – 30 Sept. 2009 | 19 Mar. 2010 – 10 Jun. 2010 |
| Rain depth (mm) | 304 | 282 | 104 |
| Restitution Coefficient (%) | 18 | 5 | 12 |
| **Total drainage volume ($m^3$; mm)** | 109192; 237 | 63052; 137 | 62203; 135 |

**Table I-4: Characterization of Bray watershed (46 ha) artificial drainage**

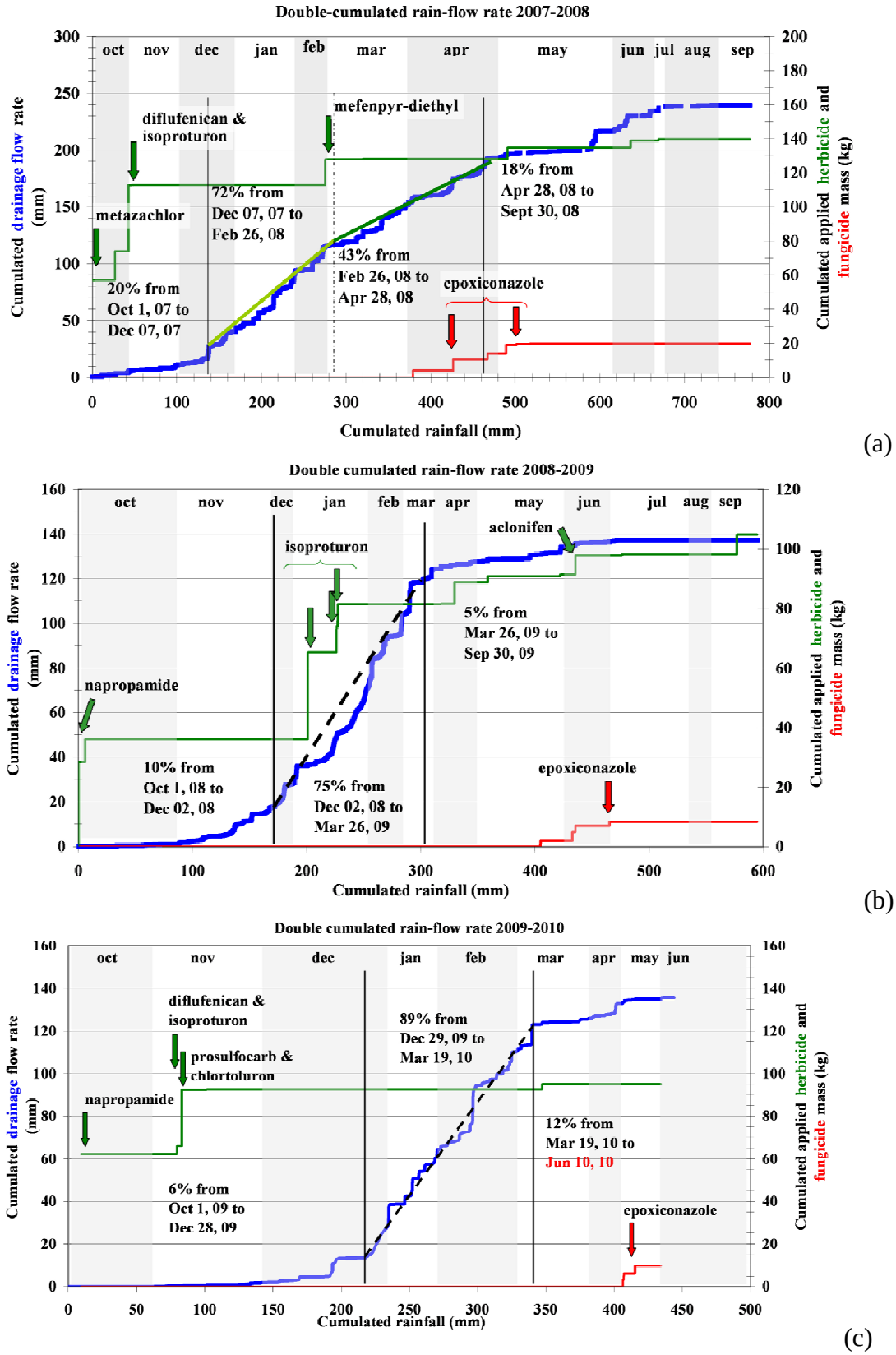



**Fig. I-12: Cumulated drainage as a function of rain volumes for the three hydrological years overlapping pesticide applications at Bray. Vertical lines indicate the separation between periods of different flow regimes whose starting and ending dates are presented together with the corresponding rainfall restitution coefficients. Applications of pesticides belonging to the analytical method are indicated with arrows. 2009–2010 hydrologic year ends on June 10, 2010.**

*4.3 Definition of the time periods assumed to correspond to the highest risk of transfer*

It was demonstrated that the first significant floods after applications are of greatest concern for pollutant transfer via pipe drainage systems thus presenting the "highest risk of transfer" (Kladivko et al., 2001; Branger et al., 2009). Generally, during a flood, most pesticide loads are exported in the first part of the flood. Subsequently, a dilution effect generates additional exportations together with much larger water volumes (Branger et al., 2009). Consequently, the part of the first floods after pesticide applications presenting the highest risk of transfer is likely to reside in the first drained water portion.

Pesticides were less employed during the intense drainage period (Fig. I-12) during which they are likely to be easily transported to surface waters as flows are frequent and intense. Overall, as early winter and spring drainage flows overlapped with pesticide application periods (Fig. I-9), these flows are likely to export high pesticide loads. The long intense 2007 – 2008 drainage period (unusually ending late April 2008) corresponded to applications thus suggesting possible pesticide losses in March and April 2008 (Fig. I-12). May 2009 presented several floods following both herbicide and fungicide applications which may transfer aclonifen and epoxiconazole (Fig. I-9 and Fig. I-12). Nevertheless, both pesticides were characterized by high adsorption coefficients ($K_{oc}$ > 1000, FOOTPRINT (2010)) and low application loads of 376 and 421 g, respectively (Appendix I). Consequently, their concentrations at the watershed outlet may be limited. Isoproturon was applied three times on Dec 10$^{th}$, 22$^{nd}$ and 23$^{rd}$ 2008 (Fig. I-9). The first flood subsequent to these applications occurred on December 18$^{th}$, 2008 and the next one was recorded on January 18$^{th}$, 2009. It is likely that the first small flood (peak flow rate lower than 8 L/s) transferred large loads of isoproturon but the transfer due to the late floods of January is rather difficult to anticipate. In 2009 – 2010, fewer floods were measured right after pesticide applications thus suggesting that pesticide exportations through the watershed may have occurred to a lower extent. However, two molecules (chlorotoluron and napropamide) were applied with high loads (25480 and 14989 g, respectively). Chlorotoluron is moderately soluble and persistant (FOOTPRINT, 2010) and may present a risk of transport associated with higher concentrations than napropamide (which had a higher (885 mL/g) $K_{oc}$) in the first floods of the hydrologic 2009 – 2010 year. In addition, the periods between napropamide application and the first peak flow (rate lower than 10 L/s, 3 months after application) and second peak flow (rate of 15 L/s, 4 months after application) are longer than those for chlorotoluron (2 weeks for the first flood and 6 weeks for the second one). Chlorotoluron field half-life ($DT_{50}$, 34 days) is of the same order of magnitude, though twice as low, as that of napropamide (72 days) (FOOTPRINT, 2010). Finally chlorotoluron GUS coefficient (2.79) is slightly greater than that of napropamide (1.94) (Table I-2). This may have prevented napropamide from transfering to the watershed outlet at a lower extent than chlorotoluron.

In order to refine the determination of time periods of concern for pesticide transfer, watershed outlet composite and some time-dependent samples were taken and analyzed.

*4.4 Pesticide transfer through the Bray catchment*

4.4.1 Larger time-scale: pesticide transfer along the year

*4.4.1.a Overview of pesticide concentrations*

The collection of flow-weighted composite samples at the watershed outlet covered the period from April 2007 to May 2010. Suspended matter (MES) and nitrate ($NO_3^-$) concentrations ranged up to 578.0 ($n$ = 90) and 82.5 ($n$ = 90) mg/L, respectively. Mean and median concentrations were 140.6 and 113.7 (MES) and 33.6 and 35.0 ($NO_3^-$) mg/L.

Pesticides for which concentrations were higher than the limit of quantification (and frequency of quantification) were isoproturon (95%), chlorotoluron (85%), metazachlor

(68%), epoxiconazole (29%), and diflufenican (22%). Similar frequencies of detection were observed for isoproturon and metazachlor at an artificially drained catchement in Sweden (900 ha) (Kreuger, 1998). Concentration ranges for the most frequently quantified molecules are presented in Fig. I-13. Observed maximal peak concentrations and associated analytical uncertainties were 88.00 ± 15.57 (isoproturon), 13.23 ± 0.40 (chlorotoluron), 4.15 ± 0.23 (metazachlor), 2.27 ± 0.13 (epoxiconazole), and 0.64 ± 0.05 (diflufenican) µg/L. None of pesticide concentrations at the watershed outlet followed a normal or log-normal distribution ($\alpha$=0.05). Departure from a log-normal distribution for the most mobile molecules (isoproturon, chlorotoluron and metazachlor) was lower than for the most sorbing molecules (epoxiconazole and diflufenican). This is explained by the latter concentration datasets presenting more numerous low values than the former, including values lower than the limits of quantification. Sorbing molecules are therefore less easily transferred through artificially drained catchments than moderately sorbing molecules. As noted previously herein and in other studies (Kladivko et al., 2001; Branger et al., 2009), pesticide concentrations were usually in accordance with farmer pesticide applications and rain flow events. Among the other pesticides that have been used on the watershed and searched for, atrazine, chlorothalonil, prosulfocarb, fenpropidin, ethofumesate, cyproconazole and aclonifen were usually not detected and diflufenican, tebuconazole and mefenpyr-diethyl concentrations were on some occasions between the limits of detection and quantification.

#### *4.4.1.b Individual pesticide concentrations along the monitoring period*

Composite sample concentrations in pesticides at the catchment outlet are presented in Fig. I-14 to Fig. I-18 for the three years of monitoring. These data confirm that the first floods following pesticide applications are those usually associated with the highest concentration values: isoproturon in the three years, chorotoluron in 2009 – 2010, napropamide with much lower concentrations in 2008 – 2009 and 2009 – 2010. This is true whatever the flow rate size and was previously reported (Kladivko et al., 2001; Schulz, 2001). For instance, it was shown that isoproturon and metazachlor highest concentrations at subsurface drained catchment outlets reflected their application patterns being the highest after their applications (Malterre et al., 1997; Kreuger, 1998; Muller et al., 2002).

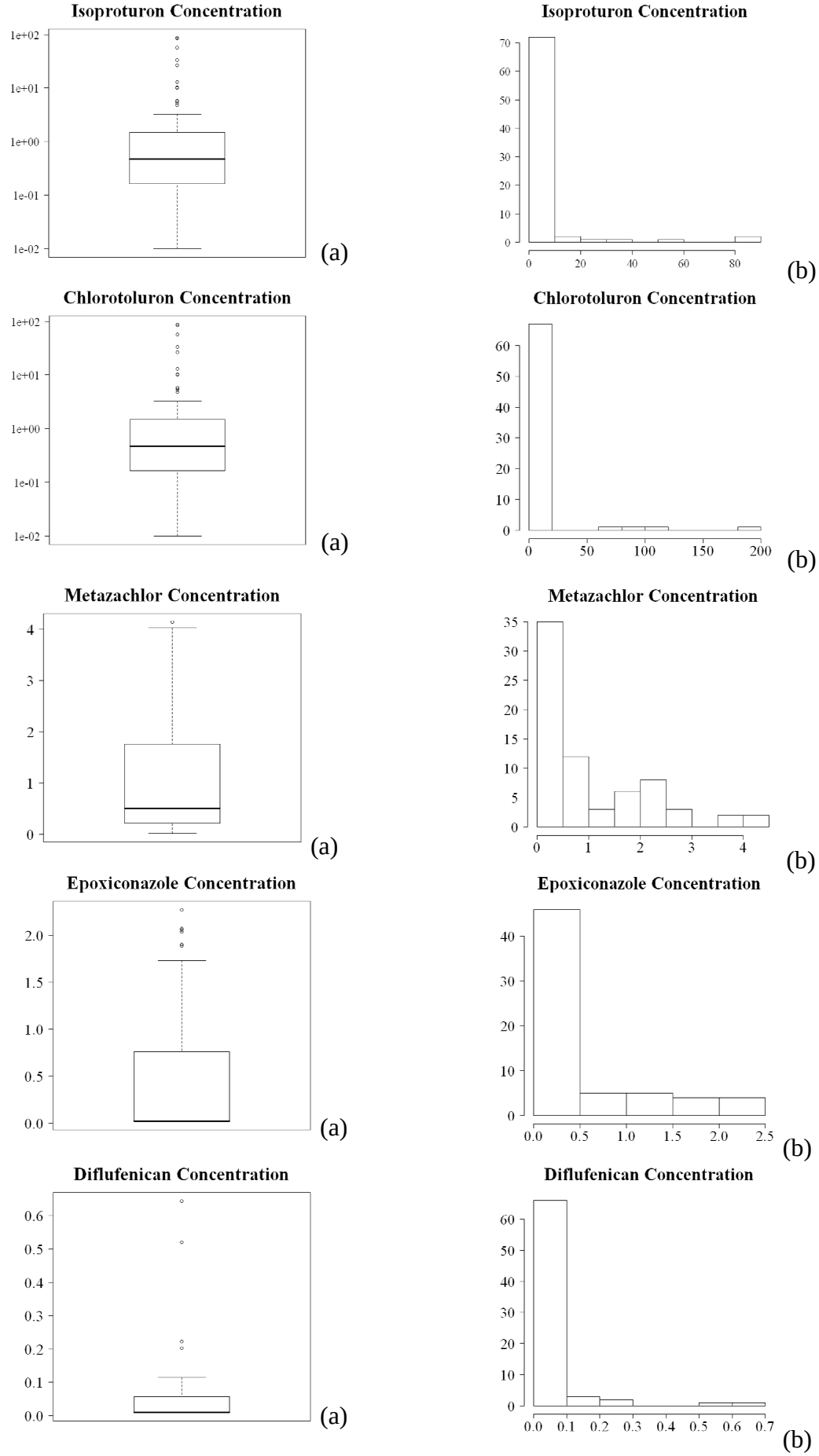



**Fig. I-13: Bray watershed outlet concentration (µg/L) distribution according to (a) box-plots and (b) histograms (frequency in %) representations.**

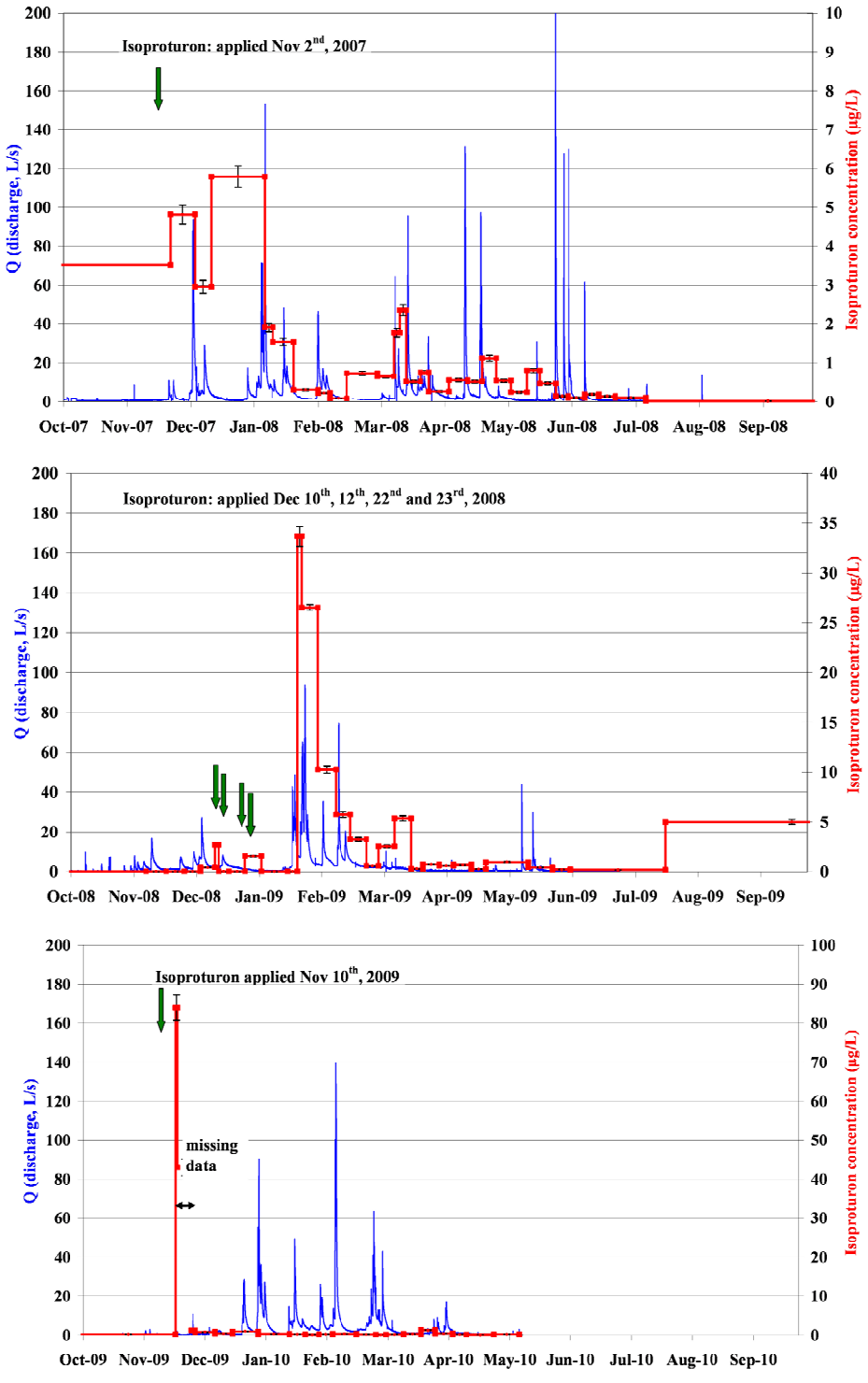



**Fig. I-14: Watershed outlet discharge and flow weighted composite samples' isoproturon concentrations. (a) 2007–2008, (b) 2008–2009 and (c) 2009–2010. Green arrows represent isoproturon (herbicide) applications.**

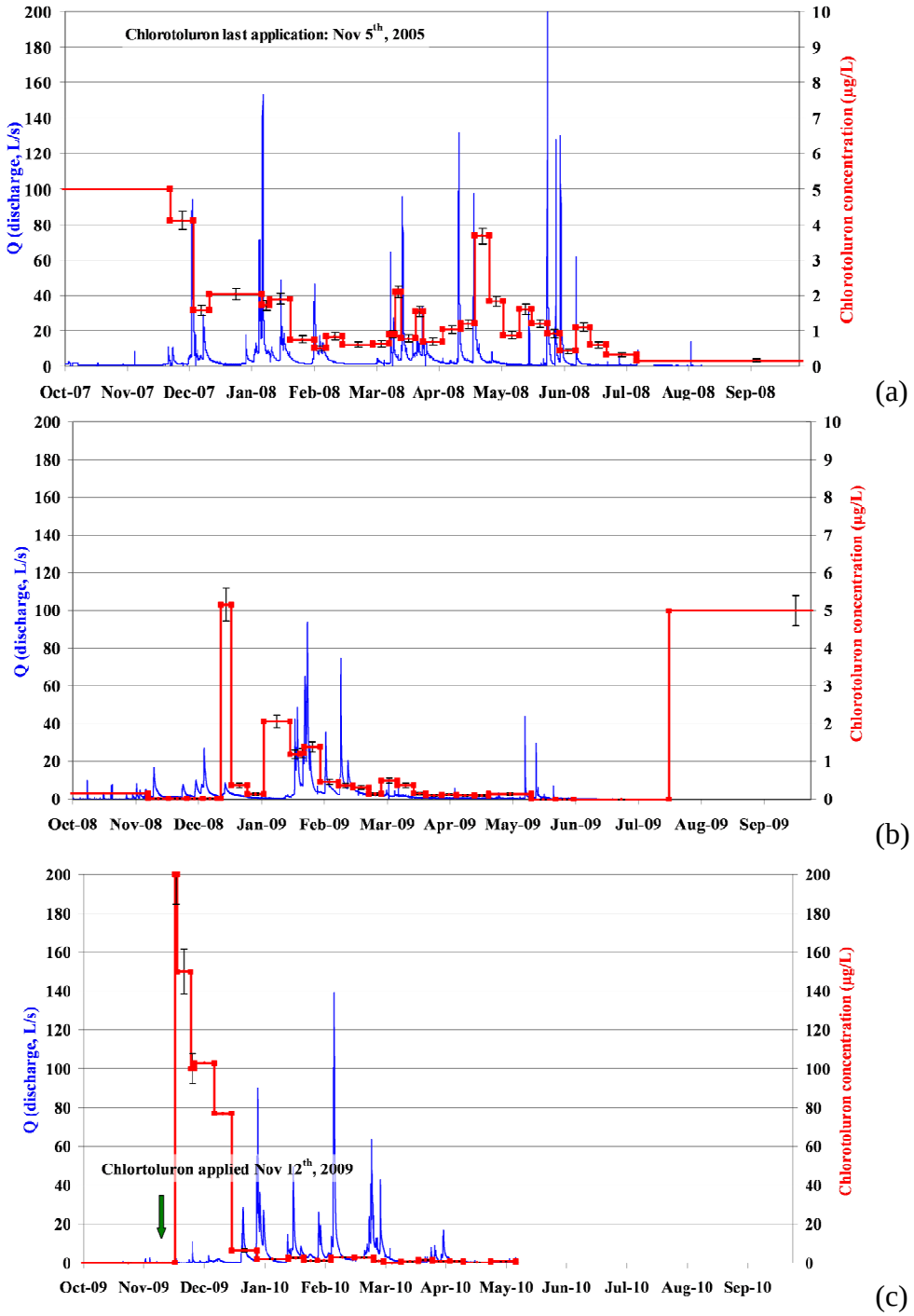



**Fig. I-15: Watershed outlet discharge and flow weighted composite samples' chlorotoluron concentrations. (a) 2007–2008, (b) 2008–2009 and (c) 2009–2010. Green arrows represent chlorotoluron (herbicide) applications.**

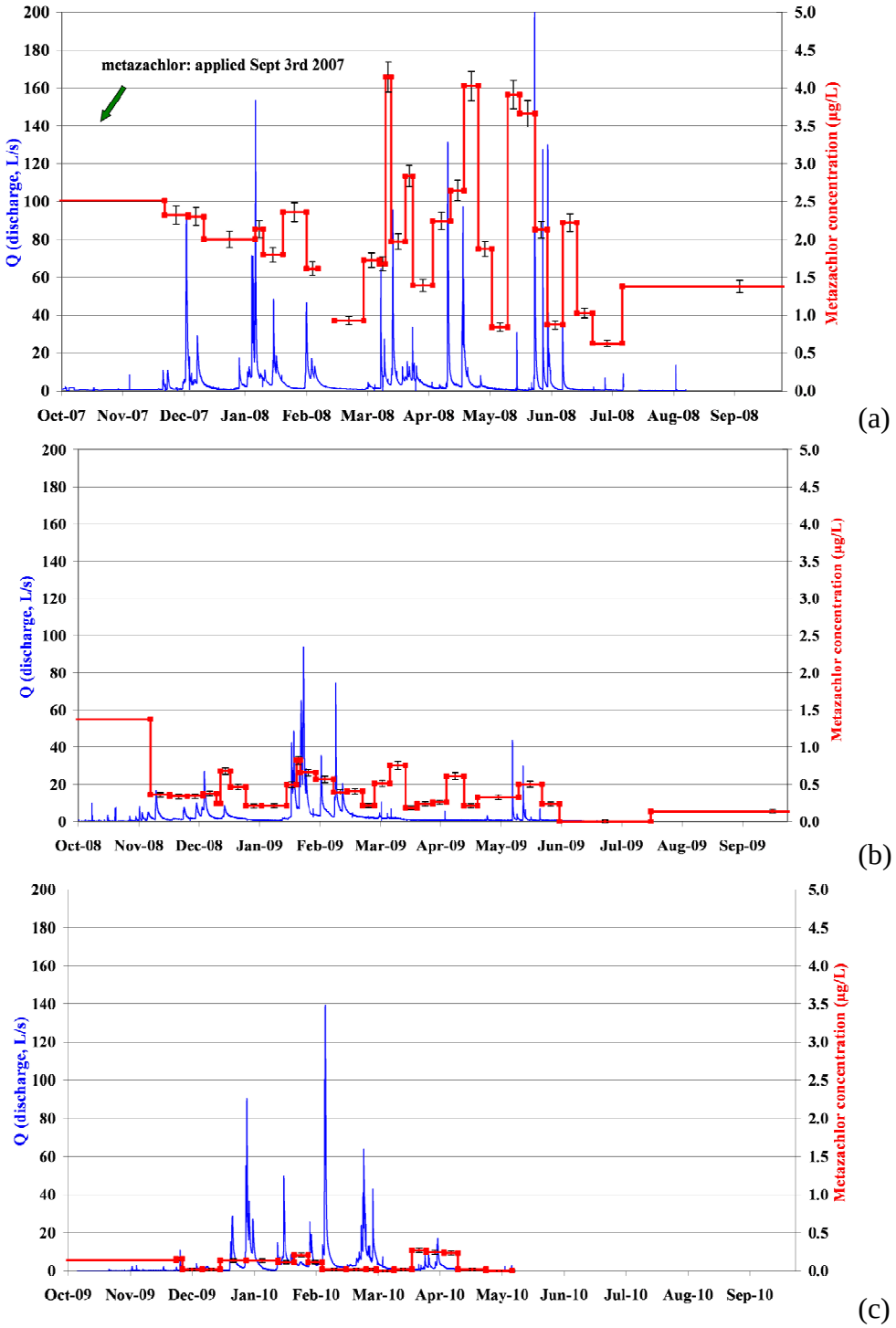
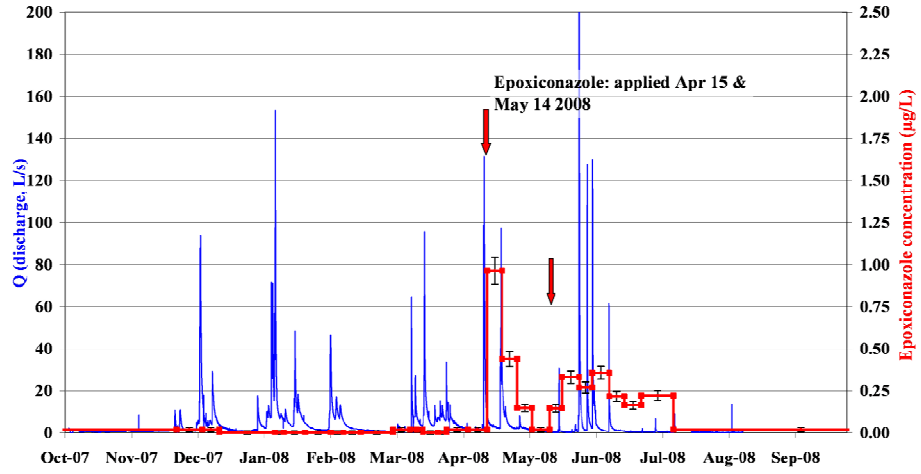
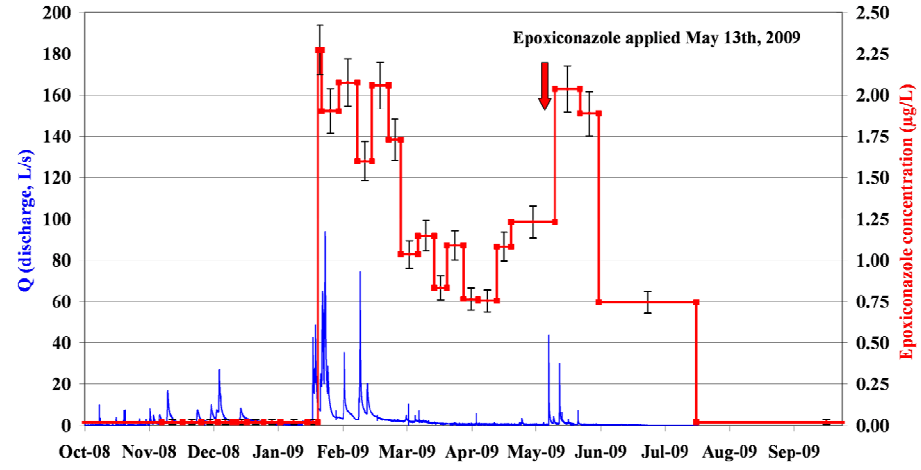


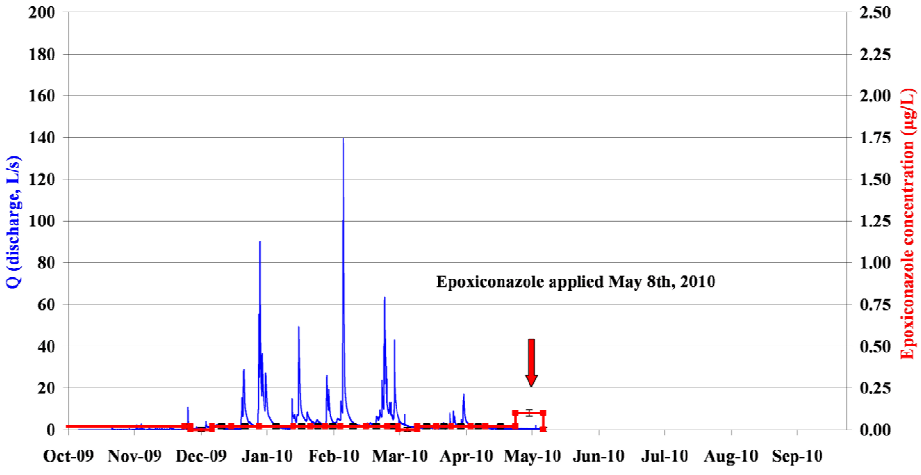

**Fig. I-16: Watershed outlet discharge and flow weighted composite samples' metazachlor concentrations. (a) 2007–2008, (b) 2008–2009 and (c) 2009–2010. Green arrows represent metazachlor (herbicide) applications.**




(a)

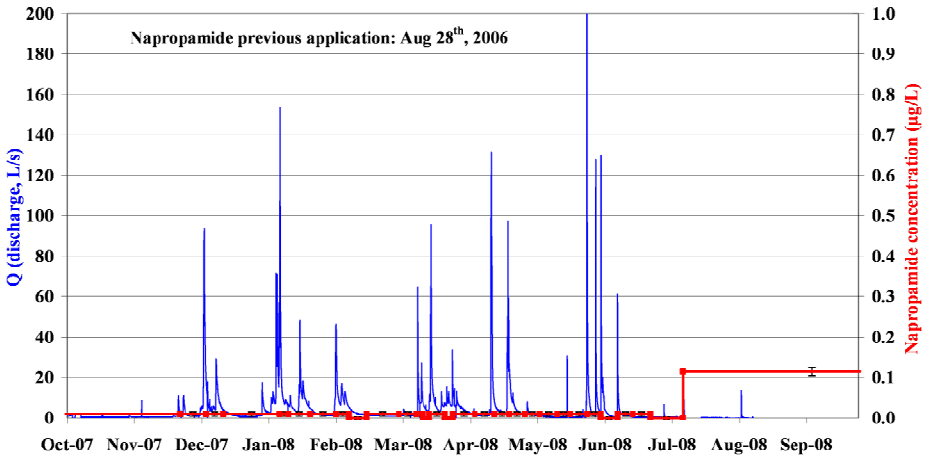



(b)

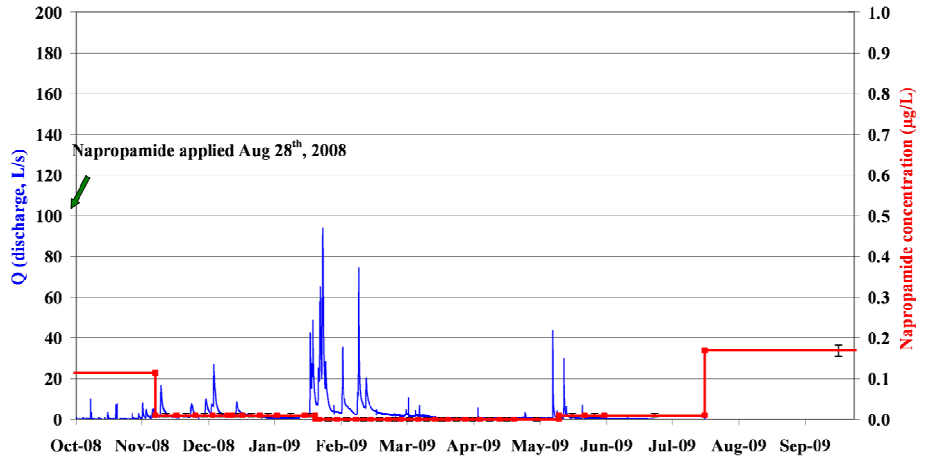



(c)

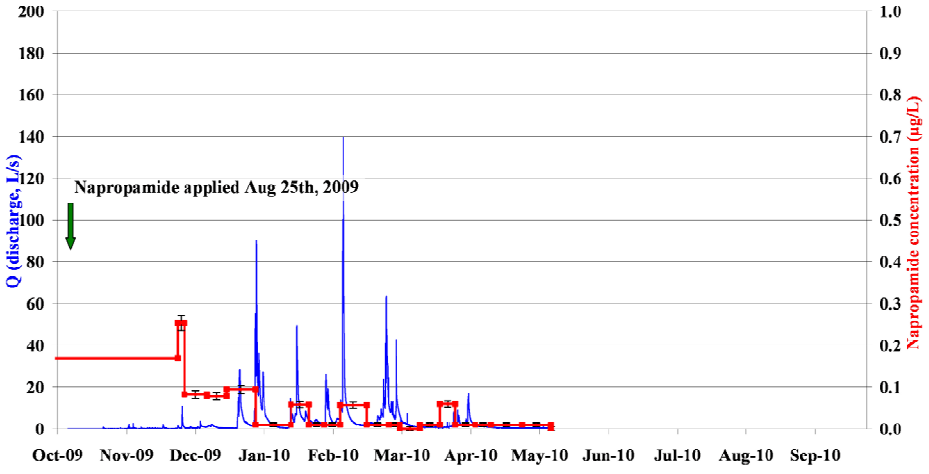
**Fig. I-17: Watershed outlet discharge and flow weighted composite samples' epoxiconazole concentrations. (a) 2007–2008, (b) 2008–2009 and (c) 2009–2010. Red arrows represent epoxiconazole (fungicide) applications.**




(a)




(b)




(c)

**Fig. I-18: Watershed outlet discharge and flow weighted composite samples' napropamide concentrations. (a) 2007–2008, (b) 2008–2009 and (c) 2009–2010. Green arrows represent napropamide (herbicide) applications.**

However, as previously discussed, floods occurring after a period of low flows may also transfer high concentrations of molecules. This was observed for instance in March 2008 for isoproturon (Fig. I-14). A period of low flow, between early February and early March 2008, also corresponding to very low temperatures, preceded a series of flows with high isoproturon concentrations. Chlorotoluron also showed concentration increase after a period of low flows in March 2008 and January 2009 (Fig. I-15).

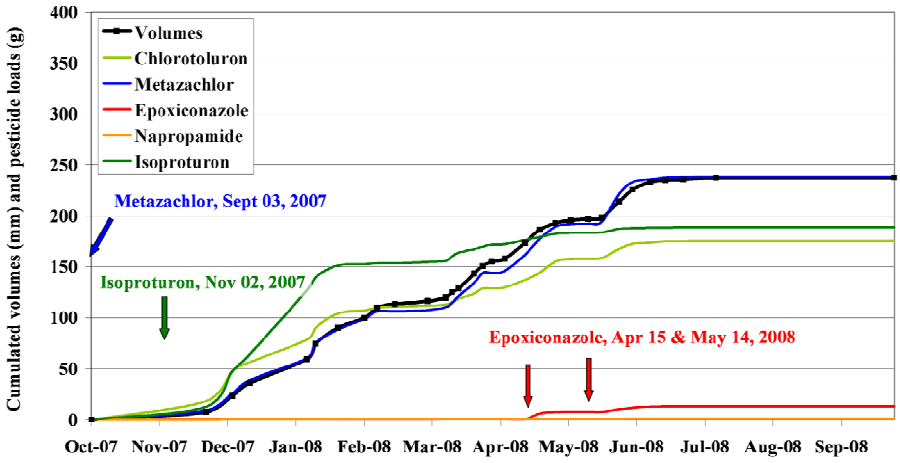
A similar behavior was noted for epoxiconazole in 2008 – 2009 (Fig. I-17). In mid-January 2008, whereas epoxiconazole previous application was 8 months earlier and 78 mm of water had passed through the catchment outlet, concentrations greater than 1.5 µg/L were observed. Muller et al. (2002) observed that pesticides with high sorbing capacities may present a delayed concentration peak compared to its application date. Only small floods occurred in the meantime that may have transferred down epoxiconazole thus giving it time to reach the drainage systems. Contrary to isoproturon or metazachlor, epoxiconazole is a strongly sorbing molecule. However, a portion of the molecule will not bind to soil. Once in deeper horizons above the drainage systems (in the first meter of the soil), the content of organic matter onto which epoxiconazole may sorb, may be less important. Epoxiconazole reaching the watershed outlet at this time could have come from that applied in between (and not above) the drains, from which it progressively reached the drain.

Metazachlor was applied only once during the three years of monitoring in 3 September 2007 (Fig. I-16). Overall, its concentration decrease from 2007 to 2010 is clear. However, metazachlor concentration pattern in the first floods following its application is more variable than that of isoproturon or epoxiconazole, with concentrations ranging from 0.5 to 4 µg/L between March and June 2008. Similarly to epoxiconazole, high concentrations of metazachlor were observed in fall 2008, on the second year after application, when the drainage started again. Kreuger (1998) also observed the highest concentrations of metazachlor at a subsurface drained catchment (900 ha) after its application. In addition, this study also highlighted that metazachlor was still detected in spring and summer following its application (8 to 12 months later) with concentrations ranging from 0.05 to 0.5 µg/L. Another increase in metazachlor concentration after thaw was noted by Malterre (1997) but the first flows following its application remained those presenting the highest concentrations.

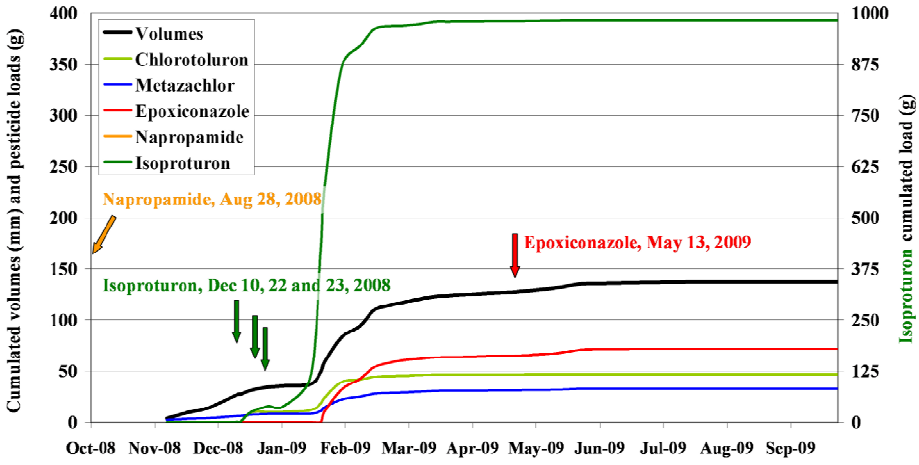
Napropamide presented low concentrations compared to the previous molecules. This may be explained by the fact that it is characterized by a fairly high sorption coefficient ($K_{oc}$ = 885 mL/g), a moderate application dose (656 g/ha on 23 ha in 2009 and 495 g/ha on 14 ha in 2009), and was applied fairly early (late august) before the start of the drain flows (Fig. I-18).

#### *4.4.1.c Pesticide loads at the catchment outlet*

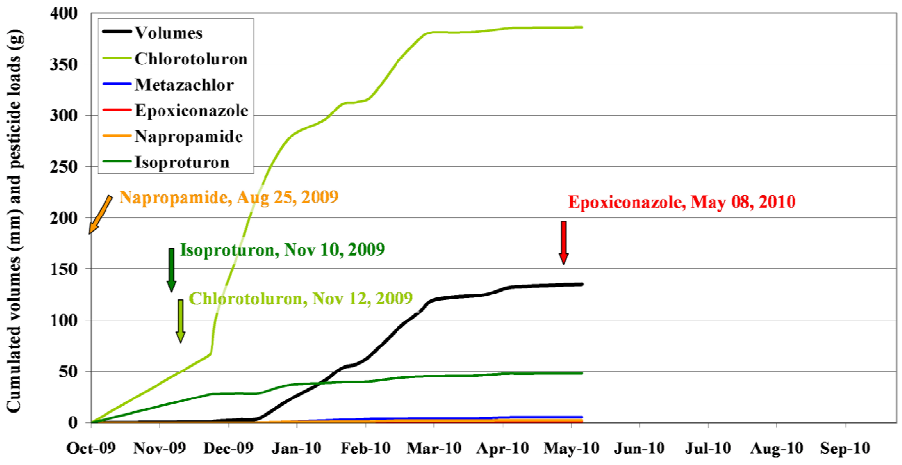
In Table I-5 to Table I-7, parts of applied masses (%) were based upon the immediately previous applied mass. For example, mefenpyr-dietyl measured watershed outlet load during the 2007 – 2008 drainage initiation period (15698 g) was compared to the 256 g applied on 12 Nov. 2006 (6.13 %). Whereas, the 57 g outlet load recorded during the intense drainage season was compared to the previously applied 1020 g on 14 ha on 21 Feb. 2008 (0.01 %).




(a)




(b)




(c)

**Fig. I-19: Cumulated volumes (mm) and pesticide loads (g) at the Bray catchment outlet for (a) 2007 – 2008, (b) 2008 – 2009 and (c) 2009 – 2010 hydrologic years. Because of large isoproturon loads exported in 2008 – 2009, the right vertical axis was used to plot isoproturon cumulated loads (g).**

Not only applied molecules were detected or quantified at the catchment outlet (Table I-5 to Table I-7). Other molecules were detected as well. Among them, no application of atrazine, fenpropidin and ethofumesate was recorded since 2002. For those that were employed by the farmer, the watershed outlet load was usually lower than 2 % of the applied mass. One exception was metazachlor for which 6.94 % of the 3435 g applied in September 2007 was exported through subsurface drainage waters in 2007–2008, i.e. the first year following its application (Fig. I-19 and Table I-5). This suggests the ease of this herbicide at being transferred towards drained catchment outlets. However, additional exported load portions subsequently decreased to 0.96 % (2008–2009) and 0.16 % (2009–2010) in the next two years while no other application occurred. Kreuger (1998) found that 0.44 % of applied metazachlor was lost from a 900 ha subsurface drained catchment. Metazachlor has similar adsorption and degradation properties as isoproturon but presents a higher solubility which may partly explain these results. Isoproturon was found to dominate pesticide loads at an agricultural catchment outlet (Muller et al., 2002) as found in our study (Fig. I-19). Isoproturon losses generally do not exceeded 4 % (Harris et al., 1995; Kreuger, 1998). It was assumed that isoproturon losses through drains resulted from soil cracks onto which it could have moved to before soil wetted up (Harris et al., 1994). Increased solute leaching due to intermittent flow (alternation between rainfall and drier periods) was explained by solute diffusion from high velocities zones in soils (ex.: cracks) during dry periods, to zones where solutes can be quickly leached when flow started again (Cote et al., 2000). 3.92 % of epoxiconazole reached the catchment outlet in 2008–2009 (Table I-6) despite being highly sorbing (Table I-2). This was due not only to 2008–2009 spread of the fungicide, but to previous applications as well. A significant portion of epoxiconazole load was exported during the intense drainage season following its application in the previous spring (Table I-6 and Fig. I-19), and during spring flows, including more than one year after being applied (Table I-5). For the other quantified and sorbing molecules (prosulfocarb, aclonifen, diflufenican), less than 1 % of applied rates were recovered during the first or first two years following their use. Very low portions of napropamide were measured at the catchment outlet (< 0.02 %) despite high application rates (> 7000 g) (Fig. I-19). This molecule has a fairly high $K_{oc}$ (885 mL/g) and moderate half-life (72 d) as shown in Table I-2 (FOOTPRINT, 2010). Mefenpyr-diethyl has a low half-life (17.5 d) which may explain its low recovery rates (< 0.06 %) at the catchment outlet. Cyproconazole was applied once in spring 2006 and recovered portion at the outlet of the Bray watershed were 0.01, 0.05 and 0.10 %, two, three and four years following this application, respectively. Although detected long after application, recovered fractions remained low. Cyproconazole has a moderate $K_{oc}$ value (309 mL/g) which may help it quickly bind to soil but may be associated to desorption (Table I-2). In addition, its half-life is high (191 d) thus conferring this fungicide a potential to be leached from the soil during extended periods of time. Overall, the highest loads, normalized to drained volumes, were recorded during drainage initiation periods. However, the spring period (end of drainage) was also associated to fairly high loads. This shows the importance of focusing in the flows following fall and spring pesticide applications, associated with high loads and low water volumes. Chlorotoluron showed high exported loads whereas it was not applied between 5 November 2005 and 12 November 2009. It seems that a stock of this molecule is slowly released each year and not totally eliminated in a one-year period.

| Molecule | 2007 - 2008 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Drainage initiation | | | Intense drainage season | | | End of drainage | | | Total | | |
| | 1 Oct. 2007 - 07 Dec. 2007 | | | 07 Dec. 2007 - 26 Feb. 2008 | | | 26 Feb. 2008 - 30 Sept. 2008 | | | | | |
| | Applied mass | Watershed outlet mass | Part of applied mass | Applied mass | Watershed outlet mass | Part of applied mass | Applied mass | Watershed outlet mass | Part of applied mass | Applied mass[(h)] | Watershed outlet mass | Part of applied mass |
| | g | mg | % | g | mg | % | g | mg | % | g | mg | % |
| Water volume (mm) | | 24 | | | 90 | | | 124 | | | 237 | |
| Isoproturon | 16992 | 47452 | 0.28 | | 106385 | 0.63 | | 34872 | 0.21 | 16992 | 188709 | 1.11 |
| Chlorotoluron | 24060[(a)] | 47672 | 0.20 | | 63470 | 0.26 | | 64242 | 0.27 | 24060 | 175384 | 0.73 |
| Atrazine | | 1810 | | | 0 | | | 75 | | | 1885 | |
| Chlorothalonil | | 0 | | | 0 | | | 3162 | | | 3162 | |
| Prosulfocarb | | 5170 | | | 3874 | | | 61 | | | 9105 | |
| Fenpropidin | | 108 | | | 57 | | | 118 | | | 283 | |
| Ethofumesate | | 6607 | | | 114 | | | 3989 | | | 10709 | |
| S-metolachlor | | 72 | | | 0 | | | 384 | | | 456 | |
| Metazachlor | 3435[(b)] | 25877 | 0.75 | | 80489 | 2.34 | | 132182 | 3.85 | 3435 | 238548 | 6.94 |
| Napropamide | 14436[(c)] | 108 | 0.00 | | 398 | 0.00 | | 459 | 0.00 | 14436 | 965 | 0.01 |
| Cyproconazole | 6044[(d)] | 0 | 0.00 | | 0 | 0.00 | | 484 | 0.01 | 6044 | 484 | 0.01 |
| Aclonifen | 12726[(e)] | 144 | 0.00 | | 0 | 0.00 | | 0 | 0.00 | 12726 | 144 | 0.00 |
| Diflufenican | 623 | 108 | 0.02 | | 648 | 0.10 | | 3060 | 0.49 | 623 | 3816 | 0.61 |
| Tebuconazole | | 7764 | | | 3913 | | | 2822 | | | 14500 | |
| Mefenpyr-dietyl | 256 | 15698 | 6.13 | 1020 | 57 | 0.01 | | 337 | 0.03 | 1020 | 16091 | 6.17 |
| Epoxiconazole | 3192[(g)] | 217 | 0.01 | | 114 | 0.00 | | 13114 | 0.41 | 3192 | 13444 | 0.42[(i)] |

**Table I-5: Applied and watershed outlet pesticide loads for the sixteen molecules quantified by the analytical method during the three identified drainage seasons in Fig. I-12 for 2007–2008. [(a)] Chlorotoluron was applied on 05 Nov. 2005. [(b)] Metazachlor was applied on 03 Sept. 2007. [(c)] Napropamide was applied on 28 Aug. 2006. [(d)] Cyproconazole was applied in spring 2006. [(e)] Aclonifen was applied on 05 May 2004. [(f)] Mefenpyr-diethyl was applied on 12 Nov. 2006. [(g)] Epoxiconazole was applied in spring 2007. [(h)] Total applied mass only accounts for pesticide applied in the 2007–2008 period. [(i)] 0.42 % includes all recovered epoxiconazole loads including the part from drainage initiation referred to previously applied mass in spring 2007 (0.01 %).**

| Molecule | 2008 - 2009 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Drainage initiation | | | Intense drainage season | | | End of drainage | | | Total | | |
| | 1 Oct. 2008 - 02 Dec. 2008 | | | 02 Dec. 2008 - 26 Mar. 2009 | | | 26 Mar. 2009 - 30 Sept. 2009 | | | | | |
| | Applied mass | Watershed outlet mass | Part of applied mass | Applied mass | Watershed outlet mass | Part of applied mass | Applied mass | Watershed outlet mass | Part of applied mass | Applied mass | Watershed outlet mass | Part of applied mass |
| | g | mg | % | g | mg | % | g | mg | % | g | mg | % |
| Water volume (mm) | | 14 | | | 110 | | | 13 | | | 137 | |
| Isoproturon[(a)] | 16992 | 63 | 0.00 | 34270 | 977834 | 2.85 | | 3579 | 0.01 | 34270 | 981476 | 2.86 |
| Chlorotoluron[(b)] | 24060 | 378 | 0.00 | | 46139 | 0.19 | | 424 | | 0 | 46941 | 0.19 |
| Atrazine | | 0 | | | 176 | | | 30 | | | 206 | |
| Chlorothalonil | | 625 | | | 3464 | | | 322 | | | 4411 | |
| Prosulfocarb | | 0 | | | 0 | | | 111 | | | 111 | |
| Fenpropidine | | 0 | | | 405 | | | 162 | | | 567 | |
| Ethofumesate | | 125 | | | 341 | | | 252 | | | 718 | |
| S-metolachlor | | 63 | | | 149 | | | 132 | | | 344 | |
| Metazachlor[(c)] | 3435 | 4113 | 0.12 | | 26805 | 0.78 | | 2034 | 0.06 | 0 | 32951 | 0.96 |
| Napropamide[(d)] | 7009 | 256 | 0.00 | | 163 | 0.00 | | 27 | 0.00 | 0 | 446 | 0.01 |
| Cyproconazole[(e)] | 6044 | 0 | 0.00 | | 2435 | 0.04 | | 489 | 0.01 | 0 | 2925 | 0.05 |
| Aclonifen[(f)] | 12726 | 37 | 0.00 | | 189 | 0.00 | 376 | 2451 | 0.65 | 376 | 2677 | 0.65 |
| Diflufenican[(g)] | 623 | 229 | 0.04 | | 572 | 0.09 | | 1349 | 0.22 | 0 | 2150 | 0.35 |
| Tebuconazole | | 125 | | | 1005 | | | 872 | | 0 | 2002 | |
| Mefenpyr-dietyl[(h)] | 256 | 19 | 0.01 | 930 | 152 | 0.02 | | 273 | 0.03 | 930 | 443 | 0.05 |
| Epoxiconazole[(i)] | 3192 | 125 | 0.00 | | 63756 | 2.00 | 421 | 8090 | 1.92 | 421 | 71972 | 3.92 |

**Table I-6: Applied and watershed outlet pesticide loads for the sixteen molecules quantified by the analytical method during the three identified drainage seasons in Fig. I-12 for 2008–2009. [(a)] Isoproturon was applied on 02 Nov. 2007. [(b)] Chlorotoluron was applied on 05 Nov. 2005. [(c)] Metazachlor was applied on 03 Sept. 2007. [(d)] Napropamide was applied on 28 Aug. 2008. [(e)] Cyproconazole was applied in spring 2006. [(f)] Aclonifen was applied on 05 May 2004. [(g)] Diflufenican was applied on 02 Nov. 2007. [(h)] Mefenpyr-diethyl was applied on 21 Feb. 2008. [(i)] Epoxiconazole was applied on 15 Apr. and 14 May 2008. [(j)] Total applied mass only accounts for pesticide applied in the 2007–2008 period.**

| Molecule | 2009 - 2010 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Drainage initiation | | | Intense drainage season | | | End of drainage | | | Total | | |
| | 1 Oct. 2009 - 28 Dec. 2009 | | | 28 Dec. 2009 - 18 Mar. 2010 | | | 18 Mar. 2010 - 30 Sept. 2010 | | | | | |
| | Applied mass | Watershed outlet mass | Part of applied mass | Applied mass | Watershed outlet mass | Part of applied mass | Applied mass | Watershed outlet mass | Part of applied mass | Applied mass | Watershed outlet mass | Part of applied mass |
| | g | mg | % | g | mg | % | g | mg | % | g | mg | % |
| Water volume (mm) | | 22 | | | 101 | | | 13 | | | 135 | |
| Isoproturon | 2501 | 36518 | 1.46 | | 8797 | 0.35 | | 2452 | 0.10 | 2501 | 47767 | 1.91 |
| Chlorotoluron | 25480 | 276625 | 1.09 | | 104320 | 0.41 | | 5301 | 0.02 | 25480 | 386246 | 1.52 |
| Atrazine | | 12 | | | 303 | | | 15 | | | 329 | |
| Chlorothalonil | | 0 | | | 2022 | | | 171 | | | 2192 | |
| Prosulfocarb | 22400 | 2039 | 0.01 | | 448 | 0.00 | | 43 | 0.00 | 22400 | 2529 | 0.01 |
| Fenpropidine | | 121 | | | 448 | | | 60 | | | 629 | |
| Ethofumesate | | 0 | | | 0 | | | 17 | | | 17 | |
| S-metolachlor | | 0 | | | 0 | | | 0 | | | 0 | |
| Metazachlor[(a)] | 3435 | 1183 | 0.03 | | 3149 | 0.09 | | 1079 | 0.03 | | 5411 | 0.16 |
| Napropamide | 14989 | 1012 | 0.01 | | 1275 | 0.01 | | 102 | 0.00 | 14989 | 2389 | 0.02 |
| Cyproconazole[(b)] | 6044 | 933 | 0.02 | | 4477 | 0.07 | | 408 | 0.01 | | 5818 | 0.10 |
| Aclonifen[(c)] | 376 | 0 | 0.00 | | 0 | 0.00 | | 0 | 0.00 | | 0 | 0.00 |
| Diflufenican | 200 | 602 | 0.30 | | 448 | 0.22 | | 60 | 0.03 | 200 | 1110 | 0.55 |
| Tebuconazole | | 0 | | | 0 | | | 86 | | | 86 | |
| Mefenpyr-dietyl[(d)] | 930 | 93 | 0.01 | | 448 | 0.05 | | 0 | 0.00 | | 541 | 0.06 |
| Epoxiconazole[(e)] | 421 | 187 | 0.04 | | 895 | 0.21 | 1073 | 157 | 0.01 | 1073 | 1239 | 0.27 |

**Table I-7: Applied and watershed outlet pesticide loads for the sixteen molecules quantified by the analytical method during the three identified drainage seasons in Fig. I-12 for 2009–2010. [(a)] Metazachlor was applied on 03 Sept. 2007. [(b)] Cyproconazole was applied in spring 2006. [(c)] Aclonifen was applied on 27 Apr. 2009. [(d)] Mefenpyr-diethyl was applied on 12 Mar. 2009. [(e)] Epoxiconazole was applied on 13 May 2009. [(f)] Total applied mass only accounts for pesticide applied in the 2007–2008 period. [(g)] 0.27 % referred to total recovered epoxiconazole load including the portion (0.04 %) from drainage initiation period.**

### 4.4.2 Fine time-scale: pesticide transfer at the flood scale

On some occasions after pesticide applications, additional automatic samplers were set up at the catchment outlet to collect time-dependent samples in separate flasks with a finer time-step than that of the flow-weighted composite samples which were collected weekly. The objective was to better characterize pesticide transfer during the first floods following their application. In addition, this led to an increased number of samples implying time-consuming and increased cost of analysis. Consequently, this was only done a few times, each of them being subsequently described in the next three paragraphs.

#### *4.4.2.a December 2008*

For being often quantified in surface waters, isoproturon is a pesticide of major concern. For that purpose, after being informed by the farmer of its imminent application, time-dependent samples were taken during 2 days after isoproturon was applied on 10 December 2008. This corresponded to the start of the intense drainage season. No major flood event occurred after the sampling equipment was set-up. As shown in Fig. I-20, a slight increase in watershed outlet flow rate ("Q_WS_out") appeared on Dec. 13th followed by a more marked flood on Dec. 15th, characterized by a peak flow of 5.7 L/s. No additional sample was taken after 15 December 2008, whereas flow rate reached back its initial background value on 23 December 2008.

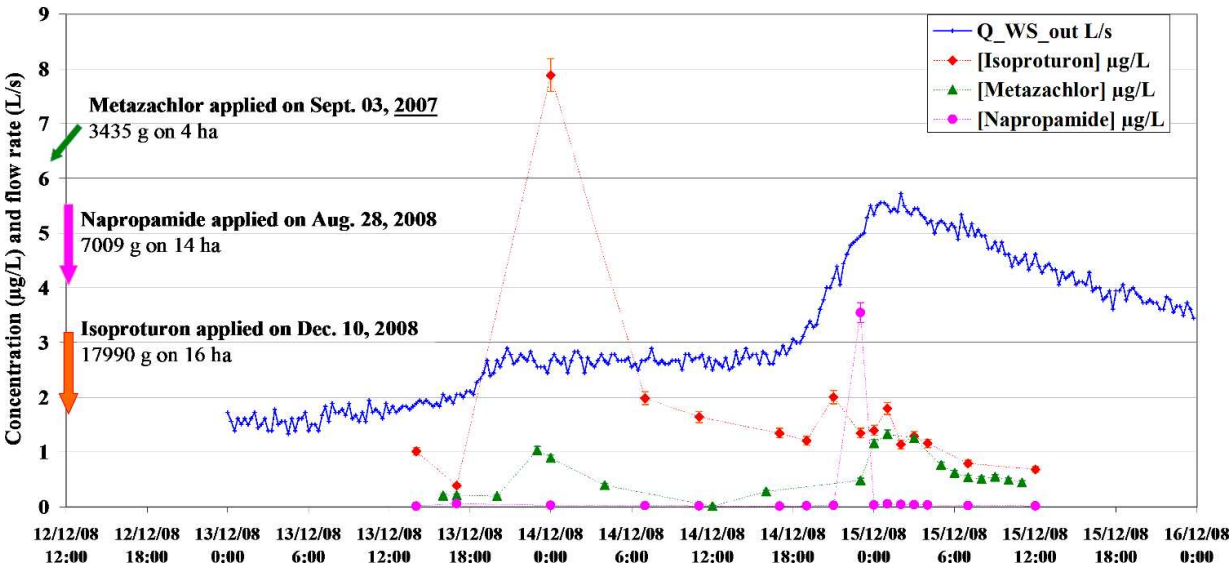
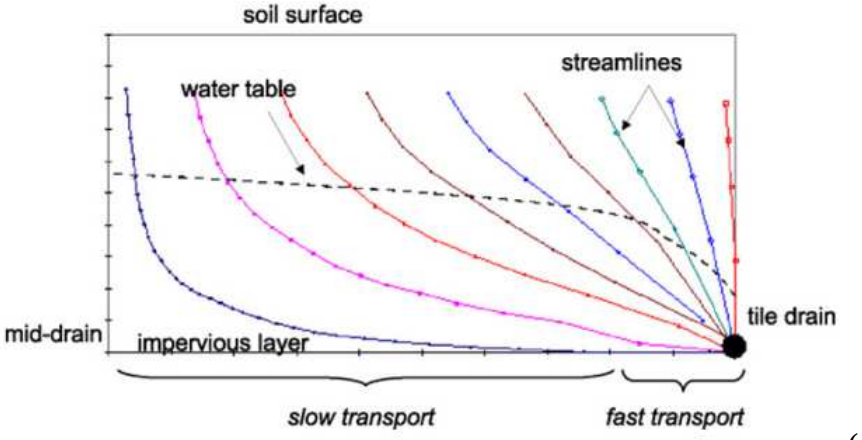



**Fig. I-20: Time-dependent samples taken at the Bray watershed outlet after isoproturon application on Dec. 10, 2008.**

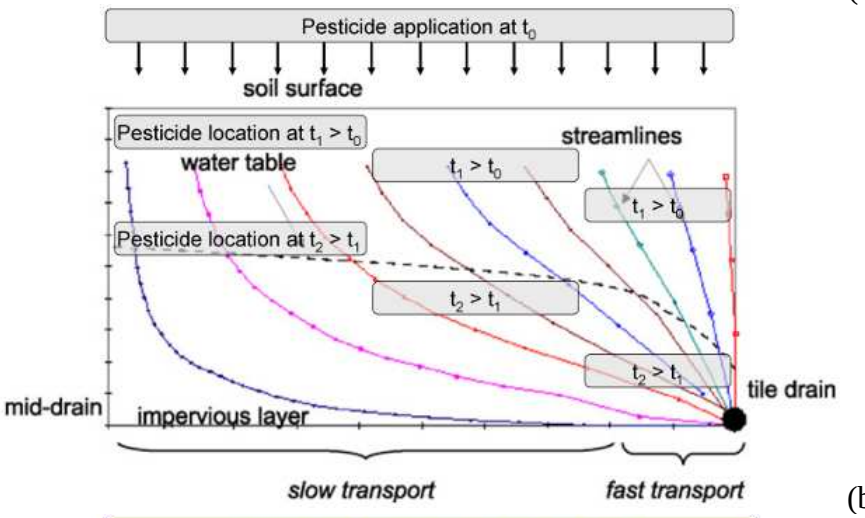
Most of isoproturon concentrations of these samples were higher than 1 µg/L. It appeared that the first slight increase in flow rate was associated with a sharp increase in isoproturon concentration suggesting that not only large flood event have the ability to transfer newly applied pesticides via subsurface drainage. Thereafter, while the flow rate stabilized at approximately 2.9 L/s, isoproturon concentrations decreased and seemed to rise up again with the next flood. From these time-dependent samples, the maximum observed concentration peak value was approximately 7.9 µg/L. A flow-weighted composite sample averaging isoproturon concentration from 11 Dec. 14:22 to 13 Dec. 11:34 had a 2.71 ± 0.15-µg/L value. The following one (from 13 Dec. 2008 11:34 to 18 Dec. 2008 17:04) was higher (approximately 13 µg/L). There is however high uncertainty (> 0.34 µg/L) on the latter value which derives from an estimate as the sample concentration exceeded the validated analytical range (0.05 – 5 µg/L) for isoproturon and could not be analyzed again after dilution. However,

it shows that high values (>5 µg/L) of isoproturon concentrations did reach the watershed outlet during this period.

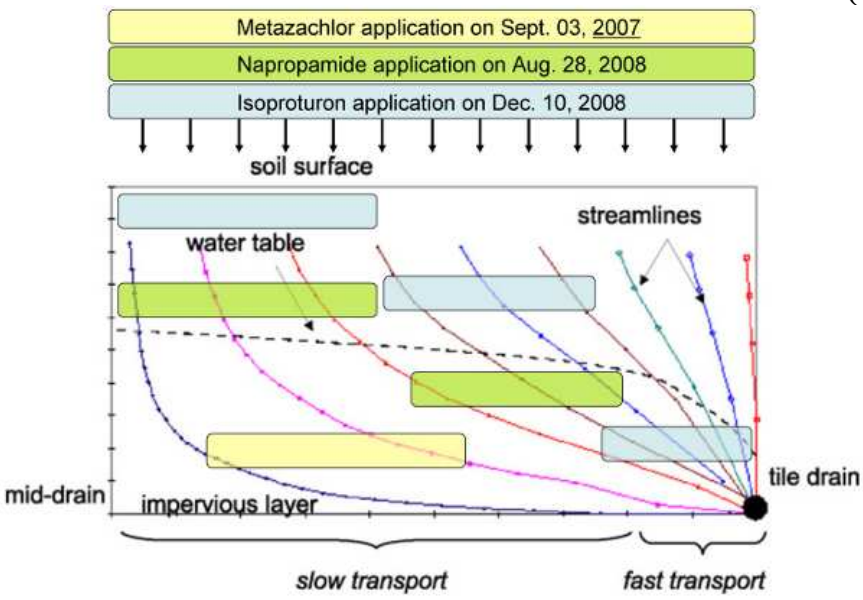
The analytical method allowing the determination of sixteen molecules simultaneously, napropamide and metazachlor concentration evolutions could also be observed together with those of isoproturon. Napropamide was applied with a lower dose (7009 g on 14 ha) than that of isoproturon (17990 on 16 ha) (Appendix III and Fig. I-20). Moreover, 3.5 months (or 12786 $m^3$ of drained water) passed between napropamide application and the analyzed flow, whereas only two days separated this flow from isoproturon application (Fig. I-20). Accordingly, lower concentrations (under the limit of quantification) were recorded for napropamide except during the more important flood of Dec. 15$^{th}$ during which one sample exhibited a high concentration (3.54 ± 0.18 µg/L). Metazachlor previous application was 15.5 months before the monitored outflow. Surprisingly, metazachlor was also quantified at fairly high concentrations ranging up to 1.33 ± 0.08 µg/L. Tests were made to verify that metazachlor was not mistaken with another compound in the analytical procedure. The two flow-weighted composite samples previously described had napropamide concentrations both lower than the limit of quantification (0.05 µg/L), while those of metazachlor were 0.24 ± 0.03 (10 to 13 Dec.) and 0.68 ± 0.05 (13 to 18 Dec.) µg/L. As noted for isoproturon, any slight increase in the flow rate seemed to generate concentration increase. These results may indicate that the herein exported metazachlor, applied much longer in advance than this event, may be located at a deeper level than isoproturon for the same event (closer to the surface). Indeed, in 15.5 months, 268 mm of water volumes passed and metazachlor, a moderately mobile and fairly soluble herbicide (FOOTPRINT, 2010), may have reached deep horizons. It may support the idea that several soil profile compartments contribute to pesticide exportations via drainage systems according to the duration between floods and their application (Branger et al., 2009). Fig. I-21 attempts to picture the assumed pesticide transfer timing and locations of the three molecules remaining loads, on Dec. 13, 2008, based on Branger et al. (2009) and Paris (2004) results. Drain outflows result from a mixture between soil water with different contribution depending on the flow rate. For large discharges, water located above the drain accounts for a large portion of drain outflows, whereas low discharges are mainly composed of water coming from the inter-drain soil portion. Fig. I-21c shows that a few days after isoproturon application, most of it is still present in the soil but isoproturon molecules have moved with different velocities through the soil profile. Above the drain, where the hydraulic gradient is the highest, isoproturon would have rapidly reached deep horizons, whereas it may remained close to the surface in the mid-drain parts of the agricultural plot. Metazachlor having been applied much longer before the pictured Dec. 13, 2008 date, loads applied above the drains have probably already been exported. Conversely, those applied in the mid-drain regions may have slowly transferred down to finally reach the drain.




(a)




(b)




(c)

**Fig. I-21: Tile-drained soil profile modified after (a) (Branger et al., 2009), representing (b) pesticide location at two dates ($t_2 > t_1$) following its application ($t_0$) and (c) proposed approximately locations of metazachlor, napropamide and isoproturon remaining loads, on Dec. 13, 2008.**

This is of high importance to determine which flows present the highest risks of pesticide transfer as it appeared here that not only the first flows following pesticide application can transfer high loads of pesticides, particularly for moderately mobile pesticides such as isoproturon and metazachlor.

### *4.4.2.b April 2009*

Epoxiconazole is a fungicide frequently applied on the Bray catchment. This molecule has a much higher adsorption coefficient ($K_{oc}$ > 1000 mL/g) than isoproturon ($K_{oc}$ = 122 mL/g) or metazachlor ($K_{oc}$ = 134 mL/g) (FOOTPRINT, 2010). It is applied in spring, whereas isoproturon and metazachlor are applied in fall. To highlight possible differences in the transfer of molecules presenting varying characteristics, the first floods following epoxiconazole application, on 13 May 2009 (421 g on 6 ha), were specifically monitored by means of time-dependent samples. However, unfortunately, the first flood occurring on May 14th, 2009, following epoxiconazole application was missed (Fig. I-22).

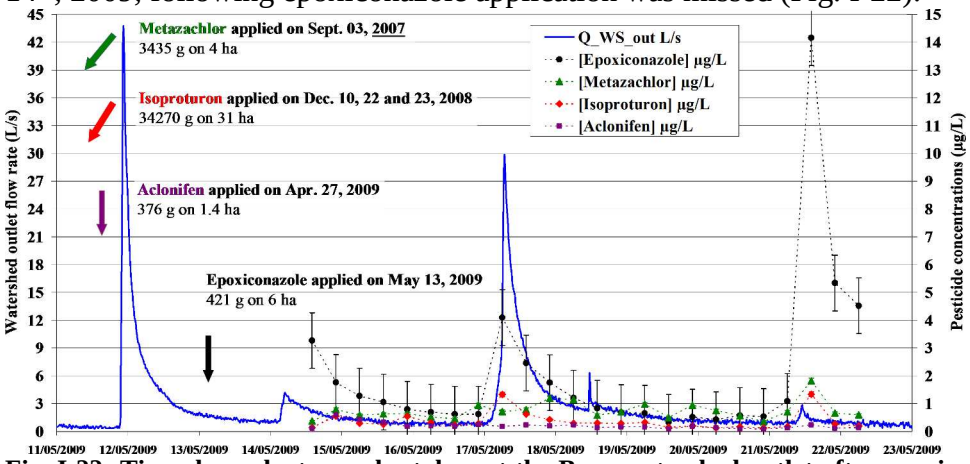



**Fig. I-22: Time-dependent samples taken at the Bray watershed outlet after epoxiconazole application on May 13, 2009.**

It should be noted that epoxiconazole was applied right after the occurrence of a large flood, on May 12th following a long period (from 20 February to 11 May, 2009, i.e. 2.5 months approximately) of low flows (Fig. I-9b). The soil of the watershed may have been refilled with water after this event. Nevertheless, from Fig. I-22 inspection, it clearly appeared that this missed flood probably transferred high concentrations of epoxiconazole, as did the second (14 May 2009), third (17 May 2009) and fourth (21 May 2009) flows. The maximum observed concentration (4.09 ± 0.21 µg/L) on May 17th was lower than that observed for a much smaller flood on May 21st (14.16 ± 3.58 µg/L). *Daphnia magna* $LC_{50}$ (48-h acute toxicity) for epoxiconazole is 8.79 mg/L, three orders of magnitude higher than the maximal recorded value. The associated toxic unit was -2.79 ("low"). Based on this criterion, such a high concentration may therefore not be harmful for aquatic living organisms present in the agricultural ditch, the artificial wetland or in the receiving stream. The “real” maximal concentration, in both cases, may have been missed depending on the way the sampling equipment was programmed. In addition, it should be noted that larger flow volumes in May 17th may have led to a higher dilution of epoxiconazole concentrations than the May 21st flow. Finally, a rapid and short moderate increase of flow rate noted in May 18th, 2009 was not associated with concentration increase. A finer time-step may have been necessary to note any possible quick phenomenon. In addition, because this flood was short and moderate, this may have been less significant than the other monitored events during this period.

Among the applied molecules that could be measured by the analytical method, metazachlor, aclonifen and isoproturon were also detected and quantified (Fig. I-22). Isoproturon showed a similar pattern, but associated to lower concentrations than epoxiconazole for May 17th and May 21st floods. Contrarily, metazachlor exhibited fairly stable concentrations, averaging 0.75 ± 0.05 µg/L, except during the last small flood which was associated with larger concentrations. Metazachlor peak concentration during this last flood (1.83 ± 0.10 µg/L) was higher than that of isoproturon (1.34 ± 0.09 µg/L). Metazachlor and isoproturon were applied 19.5 and 3.5 months, respectively, before the first flow analyzed

here (14 May 2009) (Appendix I). Before 3 September 2007 (3435 on 4 ha), the previous application of metazachlor was recorded on 12 May 2005 (4032 g on 7 ha). It seems that a stock of metazachlor was still present in the soil in spite of its moderate applied doses. Aclonifen was spread only 2 weeks before the monitored flow events presented in Fig. I-22. A low load was applied (376 g on 1.4 ha) although being similar to that of epoxiconazole (421 g on 6 ha). However, aclonifen adsorption coefficient is very high ($K_{oc}$ = 7126 mL/g) which confers an extremely reduced mobility in soils to this molecule which may also have been diluted along time with incoming rainfalls. This may explain why it was almost not detected at the catchment outlet.

| Pesticide | Period of flow-weighted composite sample composition | |
|---|---|---|
| | Concentration ± uncertainty µg/L | |
| | 25 Apr, 13:13 to 15 May, 18:44 | 15 May 18:44 to 27 May 8:38 |
| Epoxiconazole | 1.23 ± 0.10 | 2.04 ± 0.14 |
| Metazachlor | 0.33 ± 0.03 | 0.50 ± 0.04 |
| Isoproturon | 0.945 ± 0.06 | 0.43 ± 0.03 |
| Aclonifen | 1.11 ± 0.09 | 0.18 ± 0.02 |

**Table I-8: Flow-weighted composite sample concentrations and associated analytical uncertainties (µg/L) for the two samples collected during the specific monitoring period of April 2009.**

During the fine-time scale period of April 2009, two flow-weighted composite samples were collected whose concentrations are presented in Table I-8. These values are in accordance with the time-dependent ones. The first flow-weighted composite sample (collected between 25 Apr, 13:13 and 15 May, 18:44) exhibited a high concentration for the four molecules including epoxiconazole. This suggests that the first low flow occurring on 14 May 2009 may have already transferred large amounts of the fungicide. Epoxiconazole loads remaining from previous applications may also have accounted for this value (1.23 ± 0.10).

## 5 Conclusions

This first chapter showed that large water volumes were generated every year by the artificially drained watershed always exceeding 60 000 $m^3$ (130 mm). Subsurface artificially drained watersheds, as the Bray catchment, present three distinct phases centred on the intense drainage season taking place in winter. Herbicide applications represented approximately 85 % of applied pesticide loads. All pesticides together, on average, 109 kg were applied every year on the 46 ha catchment. Herbicides and fungicides are mainly applied in fall and spring when drainage flows are not as large as during the intense drainage season. Modern pesticides used in Europe usually present lower application amounts and lower leaching properties than older molecules which may minimize their potential at being transported to catchment outlet. However, from selected flood detailed monitoring, it appeared that pesticide concentrations increased with any slight flow rate increase following its application. In addition, high concentrations of some pesticides were also measured at the catchment outlet even long after their application, after the end of the drainage season. Because of limited land-availability, the total volume can not be treated by the buffer zones. Consequently, a portion of the total outflow has to be selected and forced to pass through buffer zones. In order to provide the best treatment, it was chosen to focus on the flows presenting the highest risks of pesticide transfers, and particularly those following pesticide applications. The way these specific flows were caught into the buffer zones will be described in Chapter III via what was called the "open – close" strategy.

For being quantified fairly frequently and at high concentrations, isoproturon, metazachlor and epoxiconazole were the pesticides that we focused on in our laboratory study of transfer and transformation processes in buffer zones. Their expected fate within these

systems are therefore of primary interest, and have been extensively studied in the laboratory as presented in the next chapter.

# CHAPTER II: ARTIFICIAL WETLANDS AND FOREST BUFFERS POTENTIAL FOR ADSORPTION, DESORPTION AND DEGRADATION OF SELECTED PESTICIDES

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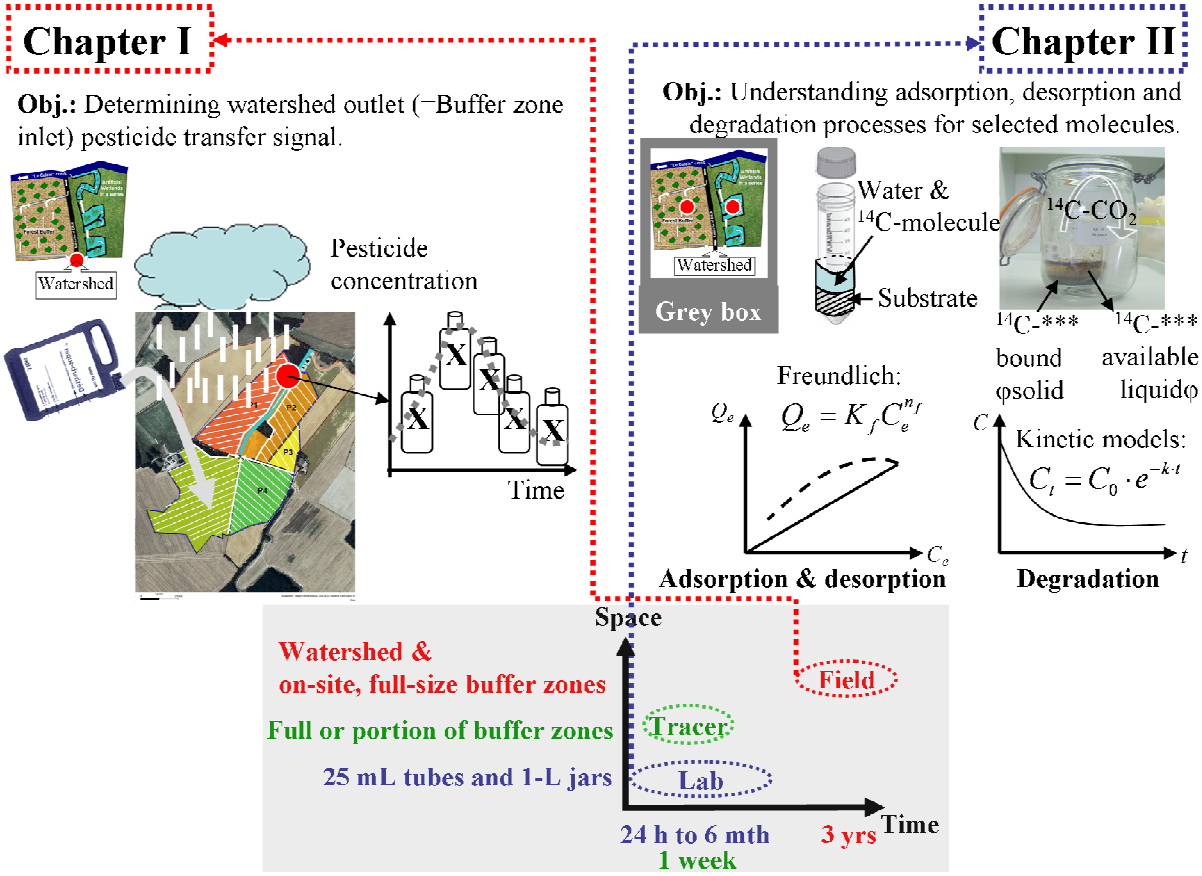


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# 1 Introduction

This chapter aims at "expressing maximal potential" of artifical wetland and forest buffer main substrates for isoproturon, metazachlor and epoxiconazole adsorption, desorption and degradation, thanks to radio-labelled molecules. Beforehand, a brief overview of literature results about these processes and molecules is presented below.

# 2 Literature review

## *2.1 Selected pesticides*

### 2.1.1 Isoproturon

Isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea, IPU) is a selective systemic herbicide belonging to the phenylurea family which inhibits photosynthesis after roots or leaves absorption. It is mainly applied at pre- or post-emergence stages to control weeds in winter wheat, winter rye and barley (e-phy, 2010). Recommended maximum applied dose is 1.2 kg $ha^{-1}$ (Couteux and Lejeune, 2008). From January 2008, isoproturon use has been greatly restricted in France on artificially drained plots (AFSSA, 2007). It is registered in Annex X of the Water Framework Directive as a priority dangerous substance requiring special attention. Isoproturon acute (letal concentration for 50% of initial population measured over a 48-h period, 48-h $LC_{50}$) and chronic (no effect concentration after a 21-day exposition, 21-d NOEC) ecotoxicities are 0.58 mg $L^{-1}$ (unknown invertebrates) and 0.12 mg $L^{-1}$ (*Daphnia magna)*, respectively (FOOTPRINT, 2010).

### 2.1.2 Metazachlor

Metazachlor (2-chloro-*N*-(pyrazol-1-ylmethyl)acet-2′,6′-xylidide, MTZ) is a selective herbicide from the chloroacetamide family which inhibits cell division and possibly the synthesis of very long chain fatty acids. It is absorbed by roots and hypocotyls to control winter and annual grasses at the pre-emergence and early post-emergence stages of various dicotyledon crops such as mustard, rapeseed, sunflower or monocotyledon like cabbage (e-phy, 2010). Maximum doses depend on target crop and range from 0.75 to 1.25 kg $ha^{-1}$ (Couteux and Lejeune, 2008). Metazachlor 48-h $LC_{50}$ is 33 and 21-d NOEC is 0.1 mg $L^{-1}$ for *Daphnia magna* (FOOTPRINT, 2010).

### 2.1.3 Epoxiconazole

Epoxiconazole ((2*RS*,3*SR*)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1*H*-1,2,4-triazole, EPX) belongs to the triazole family. It is a broad-spectrum fungicide that inhibits C-14-demethylase in sterol biosynthesis thus controlling diseases due to fungi in wheat, sugarbeet, triticale, barley, oat (Tomlin, 2003; e-phy, 2010). Epoxiconazole *Daphnia magna* 48-h $LC_{50}$ and 21-d NOEC are 8.69 mg $L^{-1}$ and 0.63, respectively.

## *2.2 Fate in the environment*

### 2.2.1 Adsorption – desorption

#### *2.2.1.a General aspects*

Most of the adsorption and desorption studies are adapted from OECD 106 guideline for individual molecules (OECD, 2000). Calvet et al. (2005a) highlighted that molecular diffusion into sediments can be a limiting step in sorption processes. Due to continuous stirring, the results observed from batch equilibrium laboratory experiments mostly reflect the adsorption part of the whole sorption process expected under on-site conditions. Indeed, in artificial wetland or forest buffer field conditions, the transport of pesticide molecules from the water column to possible adsorbent surfaces (artificial wetland sediments, plant stems or roots, forest soil components…) is limited due to convection-dispersion and molecular diffusion processes not taken into account in laboratory experiments. The soils of wetlands and forest buffers, as they are used in the present study, undergo a very wide range of moisture contents varying from dry (summer, end of the drainage season) to flooded conditions (fall, spring, winter). The alternation of aerated and anoxic/anaerobic conditions in these systems is likely to be associated to modifications in soil components, as noted for humic substances (Roy et al., 2000). These authors found that soils having low moisture contents also presented more hydrophobic surfaces. They showed that the adsorption of hydrophilic non-ionic compounds should be greater for soils with high moisture contents. This was explained by a greater ability of compounds to diffuse into soil aggregates and by a higher affinity for hydrophilic surfaces, which are more abundant in wet soils. In this study, soil moisture effect was assessed between 26.1 and 46.6 % soil moisture contents. On the other hand, Wauchope et al. (2002) recalled that dry soils and sediments may exhibit higher sorption capacity than wet soils because competition between water and pesticide molecules for soil sorption sites would be reduced. Consequently, adsorption of either polar or non-polar molecules is facilitated under dry conditions. However, their release, particularly for hydrophobic compounds, is also expected to be difficult once the soils or sediments are wetted again.

It is well-known that organic carbon helps increasing adsorption of most pesticides (Stevenson, 1972; Spark and Swift, 2002; Wauchope et al., 2002; Weber et al., 2004; Farenhorst, 2006), which is particularly true for non-anionic pesticides (Barriuso and Calvet, 1992). It is important to note that organic matter mineralization affects its sorption capacity by modifying the functional groups on its surface as observed for crop residues (Gaillardon et al., 1983; Benoit et al., 1996). Greater $K_{oc}$ values were found for several pesticides after organic matter humification (Benoit et al., 1996). The wet environments of wetlands or forest buffers are likely to help dead vegetable materials (plants, leaves) to degrade more easily than it would in a dryer environment. Another study (Arienzo et al., 1994) reported that for organic matter contents lower than 2 %, pesticide diazinon sorption was correlated to other soil properties (silt plus clay content) than the organic matter content itself. For instance, some pesticide sorption coefficients were often found to be correlated to soil clay content (Calvet, 1989; Barriuso et al., 1994; Singh, 2002; Coquet et al., 2004; Weber et al., 2004; Calvet et al., 2005a). Soil pH determines the density of electrical charges and the status of functional groups of mineral or humic surfaces which also impacts soil sorption capacities (Calvet et al., 2005a). Temperature may play a role as it affects pesticide molecular diffusion within the sorbent-solution system. Temperature decrease is known to slow down sorption velocities (Walker and Jurado-Exposito, 1998; Beulke et al., 2004). Finally, pesticide sorption

coefficients are also influenced by solid to liquid ratio. It was shown that increasing this ratio led to decreased uncertainties on sorption coefficient determination (Boesten, 1990).

### *2.2.1.b Isoproturon*

Coquet (2003) measured isoproturon $K_f$, $n_f$ and $K_d$ coefficients from 14 soil samples spatially distributed in a 187-ha agricultural catchment for which values ranges were [0.61–1.82] $mg^{1-nf}$ $L^{nf}$ $kg^{-1}$, [0.85–0.90] and [0.47–1.81] L $kg^{-1}$, respectively, for the range of equilibrium concentrations in solution [1.135–22.7] mg $L^{-1}$. Isoproturon $K_{oc}$ value ranged from 36 to 241 L $kg^{-1}$ in European commission report (FOOTPRINT, 2010). For $n_f$ values lower than unity, as also reported elsewhere for isoproturon (Nemeth-Konda et al., 2002; Boivin et al., 2005b; FOOTPRINT, 2010), sorption isotherms are classified as L- or H-shaped indicating an important affinity between isoproturon molecules and adsorbent surfaces (Calvet, 1989) but a decrease of sorption sites accessibility as concentration in solution increases. The $K_f$ Freundlich sorption coefficient indicates sorption capacity on a sorbent. Isoproturon $K_f$ on soil is relatively low (Perrin-Ganier et al., 1996; Nemeth-Konda et al., 2002) and associated to weak energy interactions with soil organic matter (Gaillardon, 1980). It was suggested that the presence of a C=O bond in isoproturon structure could help creating H–bonding and increasing its polarity (Gaillardon, 1980; Senesi, 1992; Spark and Swift, 2002). A slow equilibration between isoproturon and adsorption sites was observed by (Walker and Jurado-Exposito, 1998). Moreover, it has been showed that isoproturon adsorption was partly reversible (Nemeth-Konda et al., 2002; Spark and Swift, 2002; Boivin et al., 2005b; Benoit et al., 2008).

Organic carbon content was found to be the main variable explaining isoproturon sorption (Beck and Jones, 1996; Coquet and Barriuso, 2002; Coquet, 2003; Boivin et al., 2005b). However, it should be noted that in Coquet's study (Coquet, 2003) conducted on 14 soils, the clay content [7.6–26.9 %] and pH [7–8.5] ranges were narrow. The degree of aromaticity of soil organic matter was shown to be a good predictor of sorption of some pesticides (Ahmad et al., 2001). Benoit et al. (2008) studied isoproturon sorption on particulate organic matter from soils taken from different land uses (a forest, a grassed buffer strip and a cultivated field). Isoproturon desorption from the forest soil particulate organic matter, which presented the highest organic matter aromaticity, was also found to be the most important among these soils. Forest buffer soils may therefore enhance isoproturon adsorption but only according to a reversible process. Dissolved organic carbon fraction and soil solution ionic strength, appeared to have little effect on isoproturon adsorption (Beck and Jones, 1996; Spark and Swift, 2002). Margoum et al. (2005) compared isoproturon adsorption on dead leaves and sediments from an agricultural ditch and found that the former had a greater sorption capacity than the latter. It suggests that accumulated organic material in wetlands (dead plants and tree leaves), fresh litter in forest buffers, may play a determining role in pesticide sorption. Sorption coefficient $K_d$ was not correlated to soil pH or clay content for phenylurea compounds according to Weber et al. (2004) whose study, however, did not specifically include isoproturon. No isoproturon adsorption was found on iron oxides (Clausen and Fabricius, 2001). Conversely, Coquet and Barriuso (2002) established a relationship to predict isoproturon $K_d$ using both soil organic carbon content (positive relationship) and pH (negative relationship). Isoproturon adsorption pH dependence was also noted elsewhere (Gaillardon, 1980; Ertli et al., 2004) and explained by hydrogen bonds formation at low soil pH. For alkaline soil solutions, isoproturon may present a negative charge due to its N—H bond rupture. Consequently, repulsive forces between isoproturon and soil anionic surfaces may explain its low sorption at high pH. For soils with low organic matter content, other soil components will govern pesticide sorption. For instance, Coquet et

al. (2004) found a good correlation between isoproturon sorption coefficients and clay content for soils sampled in geological substrates presenting very low organic matter content.

### *2.2.1.c Metazachlor*

Metazachlor adsorption to soils was found to be weak (Allen and Walker, 1987; Rouchaud et al., 1996; Beulke and Malkomes, 2001). The Footprint database indicates a range for $K_{oc}$ values from 53.8 to 220 L $kg^{-1}$ (FOOTPRINT, 2010). Sorption isotherms have been characterized by $n_f$ Freundlich coefficients usually lower than unity (Allen and Walker, 1987; FOOTPRINT, 2010). Metazachlor sorption increases with time (Mamy and Barriuso, 2007). A large desorption of metazachlor was noted from soils on which its adsorption was small (Mamy and Barriuso, 2007) suggesting that it could be temporarily retained in soils or sediments. As usually noted, increase in organic matter content tends to lead to higher sorption coefficients (Rouchaud et al., 1996; Beulke and Malkomes, 2001).

### *2.2.1.d Epoxiconazole*

Many triazole fungicides, as epoxiconazole (EPX), are moderately to highly hydrophobic, thus conferring them a capacity to strongly sorb to soil (Jamet and Eudeline, 1992). These authors explained that triazole fungicides mobility decreased as their octanol to water ($logK_{ow}$) partition coefficients increased. The Footprint database reported a range for $K_{oc}$ values from 280 to 2647 L $kg^{-1}$. L-shaped adsorption isotherms are mostly observed for triazole molecules (Roy et al., 2000; Singh, 2002). However, they also noted S-shaped isotherms for hexaconazole adsorption onto two soils. Other authors also reported L-shaped adsorption isotherms for penconazole (Rodriguez-Cruz et al., 2006). EPX adsorption was usually found to adsorb to soil according to a partially reversible process (European Commission, 2006) often characterized by hysteresis as for other triazole molecules (Singh, 2002; Rodriguez-Cruz et al., 2006). The report from the European Commission however insisted in that most dissipation and accumulation studies demonstrated that EPX mobility is extremely low. Soils presenting low moisture contents may generate hydrophobic surfaces favouring the sorption of non-ionic hydrophobic fungicides like EPX (Roy et al., 2000). In addition, as noted by Wauchope et al. (2002), hydrophobic molecules adsorbed on dry surfaces may be easily desorbed after re-wetting processes. The organic carbon content was found to be a major controlling factor for triazole fungicides (Singh, 2002). Wechsler et al. (1996) studied two triazole compounds (flutriafol and flusilazol) presenting very similar properties. They found that the highest adsorption potential (Freundlich constant $K_f$) was associated to the soil with the highest organic matter content (14 %). An influence of the organic matter nature on adsorption intensity was observed through a more important adsorption of these triazole fungicides on fresh (high C/N) organic matter. Plant or leaves material in wetlands or forest systems may therefore enhance adsorption for such pesticides. Singh (2002) also noted an influence of clay and silt on adsorption constants. However, Singh (2002) and Wechsler et al. (1996) studies did not include epoxiconazole. Contrarily, the EPX European report (European Commission, 2006) did not highlight any dependency of EPX adsorption on organic carbon content, pH, and clay content. However, a high variation of the estimated adsorption coefficients was noted suggesting that additional studies should be taken into account to conclude on the effect of soil properties. Taghavi et al. (2010) found that EPX concentration was positively correlated to dissolved organic carbon and suspended matters. Although presenting high adsorption potential, triazole compounds have been frequently detected in streams (Kreuger, 1998; Berenzen et al., 2005a; Berenzen et al., 2005b) or lakes (Kahle et al., 2008). This shows that, despite its high adsorption characteristics, epoxiconazole can transfer to natural water bodies.

### 2.2.2 Degradation

In buffer zone soils or sediments, water level fluctuations imply an alternation of aerated and anoxic/anaerobic conditions. Pesticides are transferred to such systems with water resulting in inundated conditions in buffer zones under which oxygen is rapidly depleted (Mitsch and Gosselink, 2000). However, a thin layer at the soil/water interface usually presents oxidized conditions (Ponnamperuma, 1972). There is scarce literature which discusses pesticide degradation under flooded conditions. The second part of the present study focuses on laboratory experiments implemented to monitor epoxiconazole degradation under flooded conditions.

#### *2.2.2.a General aspects - Degradation under anaerobic conditions*

Under anaerobic conditions, as in wetland sediments or flooded soils, many pesticides can undergo reductive transformations (Wolfe, 1992). For example, reductive dehalogenation of aromatic compounds is generally the first metabolic step under anoxic (methanogenic, sulphate- or iron(III)- reducing) conditions (Häggblom et al., 2000) in which halogenated compounds serve as the electron acceptors. Dehalogenating microbial populations are widely distributed in anoxic environments. Populations differ according to substrate activity and specificity. Charnay et al. (2000) observed anaerobic dechlorination of 2,4-D and pentachlorophenol from a laboratory study conducted on wetland sediments. Pesticide dechlorination under anaerobic conditions led to simplified compounds more susceptible to further degradation. Wetland water level fluctuations could therefore support the action of complementary degradation processes. Pavel et al. (1999) conducted laboratory work to test the ability of riparian wetland soils from surface ponded and terrestrial horizons, as well as subsurface horizons, to degrade metribuzin and dicamba under anaerobic conditions. They concluded that wetland soils had a limited potential for the degradation of these substances. Calculated half-lives under anaerobic conditions were higher than, or in the range of, those reported in the literature for aerobic studies. However, the shortest half-life for dicamba demethylation was determined for surface soils sampled in ponded areas. While studying the ability of sequencing batch reactors technique for herbicide biodegradation, Celis et al. (2008) worked on a reactor with high inlet concentration of 2,4-D and isoproturon. For a 48-h retention time, they found that 2,4-D was degraded under both aerobic or anaerobic conditions, whereas isoproturon resisted degradation. Celis et al. (2008) also reported previous studies (Perrin-Ganier et al., 2001) in which it was postulated that isoproturon and other organic compounds containing nitrogen would not be easily degraded in nitrate-rich environments. Pavel et al. (1999) found that the presence of nitrate seemed to lead to decreased dicamba and metribuzin degradation compared to non-nitrate reducing conditions. However, dicamba does not contain nitrogen whereas metribuzin includes three nitrogen atoms.

#### *2.2.2.b Degradation of epoxiconazole*

Literature concerning EPX degradation in either soils or sediments is rather limited. According to the Footprint database (FOOTPRINT, 2010), EPX half-lives determined at the laboratory range from 98 to 649 days (typically 226 d, at 20°C) under aerobic conditions. According to the European Draft Assessment Report (European Commission, 2006) concerning EPX fate in the environment, hydrolysis is negligible. It was found that it could be partly microbially degraded (Patil et al., 1988; Tomlin, 2003). Wetlands and forest buffers are characterized by fluctuating water level which affects dissolved oxygen concentration as well as redox potentials. The redox potential was shown to present contrasting effects on pesticide biodegradation according to pesticide molecular form (DeLaune et al., 1997; Seybold et al., 2001; Boivin et al., 2005a).

*2.2.2.c Epoxiconazole degradation under aerobic conditions*

Epoxiconazole mineralization was found to be very low (< 10 % of initially applied $^{14}$C-EPX) under aerobic conditions (European Commission, 2006). Bromilow et al. (1999a) studied EPX degradation under laboratory conditions on a clay loam and a sandy loam under three temperatures (5, 10 and 15 °C) and three soil moisture contents (60, 80 and 100 % field capacity). Overall, they observed very slow degradation rates (half-lives > 2 years) but higher half-lives from the clay loam soil than the sandy loam soil. Buerge et al. (2006) estimated EPX half-lives between 2.5 and 6 months on loam or sandy loam soils incubated under aerobic conditions. Additional field trials conducted by Bromilow et al. (1999b) also reported long (> 400 days) EPX half-lives, but lower than those observed in the laboratory. They suggested that surface-loss processes like volatilization and/or photolysis may have taken place after EPX application to soil. As usually highlighted, they also noted degradation rates increasing with temperature. Other field studies Montfort et al. (1997) demonstrated that EPX, among other fungicides, was very persistant in soils probably due to an elevated sorption coefficient and slow degradation rate. Patil et al. (1988) studied the degradation of benzyltriazoles in soils and showed a good fit of first-order reaction kinetics after an initial lag-phase. They demonstrated that microbial activity had an important influence on the degradation of these compounds. 1-(4-fluorobenzyl)triazole was less affected by soil water content and temperature than the unsubstituted benzyltriazole. In addition, the latter was less lipophilic and strongly adsorbed on soil, and more rapidly degraded (14 days at 20 °C) than 1-(4-fluorobenzyl)triazole (160 days at 20 °C). Compounds containing a triazole group, as does EPX, are very persistant in the environment, although 1,2,4-triazole is very soluble in water (730 g $L^{-1}$ for 1,2,4-triazoles, 20°C) (FOOTPRINT, 2010). Buerge et al. (2006) showed that epoxiconazole degradation was enantioselective in alkaline or slightly acidic soils. They found larger half-lives of EPX for slightly alkaline soils than for acidic soils. A faster removal of one of EPX *cis*-stereoisomer was noted in these soils leading to residues' enrichment in the corresponding EPX enantiomer.

*2.2.2.d Epoxiconazole degradation under anaerobic conditions*

In the European commission draft assessment report of EPX (European Commission, 2006), anaerobic (soil under oxygen-free atmosphere) and water/sediment (flooded sediments) studies showed that EPX mineralization was very low with less than 5 % of initially applied $^{14}$C-EPX transformed into $^{14}C$-$CO_2$. In the study conducted under flooded conditions, traces of an alcohol and an alkene were detected after 120 days of incubation. These molecules were suspected to be produced after the cleavage of EPX oxirane ring successively leading to BF 480-alcohol and BF 480-entriazol alkene, as already reported by Buerge et al. (2006). However, they only accounted for a very small part of the initial radioactivity, reaching 1.2 (BF 480-alcohol) and 8.6 (BF 480-entriazol alkene) % of applied radioactivity after 120 days of incubation. A water/sediment study led to the conclusions that the alkene metabolite accumulated up to a maximum of 5.7 or 32.7 % of applied radioactivity after 100 days for a clay loam soil and a sandy soil, respectively. The study under anaerobic conditions in the EPX draft assessment report indicated the formation of 24.2 % of initial radioactivity (fluorophenyl $^{14}$C-labelled) as bound residues. Bound residues accounted for approximately 20 % in a water/sediment study of this report. They also reported that the bound residues could be allocated to the humic substances to which EPX residues were tightly bound. In a study under tropical conditions, Lin et al. (2001) found that EPX half-lives varied from 20 to > 97 days in a paddy rice soil mesocosm. Contrary to what was reported in the European Union draft assessment report, they suggested that photodegradation could partly

explain EPX degradation. To our knowledge, the Lin et al.'s (2001) study was the only one found dealing with EPX degradation at the field-scale in a wetland-assimilated system.

#### *2.2.2.e Wastewater treatment plant efficiency for triazole pollution reduction*

Although a wide range of removal efficiencies was found for several azole compounds, they are overall not significantly affected by common wastewater treatment plants. Stamatis et al. (2010) found low removal rates for some azoles fungicides (cyproconazole, tebuconazole, penconazole, triadimefon, pyrimethanil) from a wastewater treatment plant including several steps under both aerobic and anaerobic conditions. They indicated that adsorption to suspended solids and further sedimentation was one of the main ways of dissipation for the more lipophilic compounds. Indeed, no additional removal was found through aerobic and anaerobic treatment stages. Similarly, Kahle et al. (2008) observed that propiconazole and tebuconazole were almost not eliminated through wastewater treatment and were detected in Swiss lakes. Contrarily, an azole pharmaceutical, clotrimazole was effectively removed from wastewaters. Kupper et al. (2008) studied pesticide concentration evolution during open windrow composting and semi-dry thermophillic anaerobic digestion at full-scale plants, for 271 molecules among which triazoles were either slightly, or not, dissipated.

## 3 Objectives

In agricultural watersheds, pesticides are transported from crop plots to natural water bodies. The conclusions of our literature study (Introductory chapter) suggested that wetlands or forests as engineered natural buffers could help decreasing pesticide surface water pollution coming from the outlet of agricultural watersheds. However, little information exists about the mechanisms that govern pesticide fate in such buffer zones. From a surface water quality perspective, among possible removal or dissipation processes, pesticide retention and degradation could both act to remove pesticide from water. Retention refers to pesticide transport (by molecular diffusion and/or convection) and adsorption (surface phenomenon). This latter process requires that pesticide molecules reach sorbent surfaces. Pesticide degradation usually concerns forms present in solution, i.e. unadsorbed or desorbed. Several degradation processes exist, namely, microbial, photolytic and hydrolytic degradation. Microbial degradation requires the presence of microbial populations which growth partly depends on the organic matter present in their living environment. In artificial wetlands, sediments and plants are both in extended contact with water and pesticides, according to the flow regime. Similarly, in forests, both the forest soil and accumulated litter (dead leaves) can be thought as possible sorbents for pesticide molecules. Such substrates can represent potential surfaces onto which pesticide molecules can be sorbed. They can also support the growth of microorganisms that may be put in contact with contaminated water. It was previously shown that among the applied pesticides, isoproturon, metazachlore and epoxiconazole were frequently detected and quantified at the outlet of the Bray watershed. Laboratory experiments were thus conducted to give clues for better understanding of the fate of these three molecules in the studied buffer zones.

Three questions could be raised:

(1) Which of the substrate characteristics mainly govern pesticide dissipation processes? The identified substrates (wetland sediments and plants, forest soil and litter) were therefore sampled from the Bray buffer zones and complemented with wetland sediments from another artificial wetland whose characteristics differed from those of the Bray wetland sediments.

Their dissipation capacities were compared based on their major physico-chemical characteristics.

(2) To which extent would sorption play a role in pesticide retention?

In order to determine intrinsic adsorption capacities of each substrate, the batch equilibrium method was selected. As adsorption is not necessarily an irreversible process, desorption was also assessed. Reversible sorption implies a temporary removal of pesticide pollution from surface waters. However, it could also be conferred two positive aspects. First, reversible adsorption helps delaying pesticide transfer and reducing pesticide concentration peaks at the outlet of a buffer zone. Second, as molecules are released in water, they may be more easily prone to degradation.

(3) To which extent would degradation remove pesticides from water?

Pesticide degradation is obviously the major process one expects in wetlands. However, it is important to note that total degradation (mineralization) from an initial parent compound is a long process that may occur through different successive degradation stages. A parent compound will successively lead to the formation of several metabolites having their own characteristics. These newly produced molecules may differ in terms of adsorption, desorption, degradation or ecotoxicological properties from the parent compound. Their accumulation in the buffer zones is therefore a possible issue that needs to be quantified when assessing buffer zones for pesticide pollution mitigation. Artificial wetlands and forest buffers, herein considered as possible treatment systems whose efficiency is assessed, are frequently flooded by water coming from the agricultural watershed. On-site, there is an alternation between aerobic and anaerobic conditions. To approach on-site flooded situations, degradation experiments were conducted as water/sediment studies. Epoxiconazole being a frequently quantified pesticide for which little research has been done, it was selected for our degradation study.

Conducted under controlled conditions, the aim was to express the maximal potential that could be attributed to wetlands or forest buffers for pesticide mitigation. It was not intended to reproduce exactly what could occur on-site (large time and space scales). Indeed, adsorption – desorption experiments were done under small time (24 h) and space (20 mL tubes) scales, whereas incubations were carried out under small space (1 L jars) but intermediate time (6 months) scales.

## 4 Artificial wetlands and forest buffers potential for adsorption and desorption of selected pesticides

*This part is in preparation to be submitted for publication in link with lager scale results, possibly to Ecological Engineering.*

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*4.1 Abstract*

Buffer zones like artificial wetlands and forest buffers may help decrease non point source pesticide pollution from agricultural catchments. This paper focuses on understanding the role of the main substrates found in such buffer zones for pesticide adsorption and desorption. Radio-labelled $^{14}C$ isoproturon, metazachlor and epoxiconazole were used to measure adsorption and desorption isotherms on wetland sediments and plants, and forest soil and litter from two sites in France. Wetland sediments and forest soil exhibited the most important potential for pesticide adsorption. Wetland plants and forest litter also showed high adsorption coefficients and were associated with highly hysteretic desorption, particularly for the moderately mobile isoproturon and metazachlor. Contrarily, adsorption of the highly hydrophobic epoxiconazole was strong and associated with weak desorption from all substrates. Results showed that forests and wetlands present potential for pesticide retention which may be enhanced in wetlands by introducing plants in their design.

**Capsule**: Artificial wetlands and forest buffers substrates help decreasing pesticide pollution through adsorption – desorption processes.

**Keywords**:
Buffer zones, microcosms, pesticides, pollution, mitigation

*4.2 Introduction*

Isoproturon and metazachlor, two moderately hydrophobic herbicides, and epoxiconazole, a more hydrophobic fungicide, are frequently used on cereal crops and quantified at the outlet of artificially drained catchments (Garmouma et al., 1997; Kreuger, 1998). Buffer zones, like vegetative filter strips, artificial wetlands or forest buffers, may be implemented to control pesticide pollution in complement to other mitigation measures (Lacas et al., 2005; Reichenberger et al., 2007; Gregoire et al., 2008). However, the range of efficiencies can be extremely wide varying from "negative reduction" to 100 % pollution abatement. Most studies dealing with pesticide pollution reduction in wetlands or forest buffers refer to on-site comparisons of inlet versus outlet pesticide concentrations or loads under real or simulated conditions (Moore et al., 2000; Schulz et al., 2003a; Blankenberg et al., 2007). Besides such “black boxes” approaches, a limited number of studies assessed which processes govern pesticide fate in such treatment systems. While crossing artificial wetlands and forest buffers, water and pesticides interact with wetland sediments and plants, and forest soil and litter (dead leaves). Due to the presence of such substrates, adsorption is suspected to be a primary process governing pesticide fate in such systems. A better understanding of pesticide mobility in artificial wetlands and forest buffers could further help optimize their design.

It was demonstrated that adsorption increases with organic matter content (Karickhoff et al., 1979; Wauchope et al., 2002). Modifications of environmental physico-chemical conditions (eg. changes in temperature, oxydo-reduction state, water chemistry) lead to a new equilibrium that may generate further release of previously sorbed molecules. This refers to desorption. Studying adsorption reversibility is fundamental to predict pesticide mobility in aquatic ecosystems. It may help determining if artificial wetlands and forest buffers can be considered as permanent sinks or temporary reservoirs for pesticides.

Few papers reported adsorption and desorption potential of wetland sediments and plants as well as forest soil and litter, for isoproturon, metazachlor and epoxiconazole. The soils of wetlands and forest buffers undergo a very wide range of moisture contents thus associated with soil components modification, as noted for humic substances (Roy et al., 2000). These authors showed that the adsorption of hydrophilic non-ionic compounds should be greater for soils with high moisture contents. This was explained by a greater ability of compounds to diffuse into soil aggregates and by a higher affinity for hydrophilic surfaces, which are more abundant in wet soils. Charnay et al. (2000) observed that reducing conditions, as found in temporarily flooded buffer zones, slightly enhanced isoproturon adsorption to wetland sediments and led to larger fractions of non extractable residues. Isoproturon adsorption was found to be more important, but also more reversible, on forest soil compared to soils from a grass buffer strip and a crop field (Madrigal et al., 2007). Margoum et al. (2006) showed that in agricultural ditches, bottom sediments exhibited a lower sorption capacity for isoproturon than accumulated dead leaves. Dead organic material found in forest buffers or artificial wetlands could therefore play an important role in the control of pesticide pollution. On soils from agricultural areas, metazachlor sorption studies only showed weak and reversible adsorption (Beulke and Malkomes, 2001; Mamy and Barriuso, 2007); whereas epoxiconazole was found to sorb strongly (Jamet and Eudeline, 1992). No study on adsorption – desorption was carried out for metazachlor or epoxiconazole on wetlands or forest substrates. Through laboratory experiments, the objectives of this paper were to characterize the sorption and desorption potential of wetland sediments, wetland plants, forest soil and forest litter for isoproturon, metazachlor and epoxiconazole in order to improve the understanding of their fate in such buffer systems.

## *4.3 Material and methods*

### 4.3.1 Sorbing substrates

Five substrates possibly in contact with pesticides (Table II-1) were taken from buffer zones at two research sites in France. At Bray, sediments (SB) and plants (P) were taken from an artificial wetland, and soil (SF) and dead leaves (F) were sampled at a forest buffer. The two buffer zones were described previously by Passeport et al. (2010b). At Aulnoy, sediments (SA) were also taken from an artificial wetland. These five substrates were composite samples made of subsamples taken at different locations in the buffer zones. Six sediment subsamples were taken in the Bray artificial wetland (n=6), n>4 soil and leaves subsamples were taken between the forest inlet and outlet, and n>4 locations from the Aulnoy artificial wetland where the water level did not exceed 70 cm were sampled. Forest soil and wetland sediments were taken within the 0 – 10 cm surface layer. The plant substrate was a mixture of *Glyceria maxima* (71 %), *Festuca arundinacea* (16 %) and *Phragmites australis* (13 %). Wetland sediments (SB and SA) and forest soil (SF) were sieved to 2 mm. Plants and leaves were crushed and sieved to 5 mm.

| Substrate | Texture | pH | Clay (0–2 µm) ($g\ kg^{-1}$) | Silt (2–50 µm) ($g\ kg^{-1}$) | Sand (50–2000 µm) ($g\ kg^{-1}$) | OC[(a)] ($g\ kg^{-1}$) | $CaCO_3$[(b)] ($g\ kg^{-1}$) | N ($g\ kg^{-1}$) | C/N (-) | CEC[(c)] ($cmol(+)\ kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| SB | silty clay loam | 7.38 | 377 | 571 | 47 | 19.4 | 4.7 | 1.77 | 11 | 13.0 |
| SA | loam | 8.10 | 226 | 401 | 311 | 16.2 | 57.9 | 1.16 | 22 | 12.4 |
| SF | silty clay loam | 6.75 | 284 | 545 | 170 | 69.1 | <1.0 | 4.11 | 17 | 27.3 |
| P | - | - | - | - | - | 402.7 | - | 8.17 | 50 | 15.5 |
| F | - | - | - | - | - | 279.8 | - | 12.60 | 23 | 50.3 |

**Table II-1: Characteristics of the substrates studied. [(a)] OC, soil organic carbon content, [(b)] $CaCO_3$ content, [(c)] CEC, cationic exchange capacity.**

### 4.3.2 Pesticides

Isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea, IPU), metazachlor (2-chloro-*N*-(pyrazol-1-ylmethyl)acet-2′,6′-xylidide, MTZ) and epoxiconazole ((2*RS*,3*SR*)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1*H*-1,2,4-triazole, EPX) were selected for being used at the research sites. Main characteristics are given in Table II-2. $^{14}$C-labelled IPU and EPX were purchased from Izotop (Budapest, Hungary) and $^{14}$C-labelled MTZ was obtained from BASF (Limburgerhof, Germany). Unlabelled molecules were obtained from Sigma-Aldrich (St Quentin Fallavier, France).

### 4.3.3 Adsorption and desorption experiments

Adsorption and desorption experiments adapted from the standard batch equilibrium method (OECD, 2000) were conducted on the 5 substrates for the 3 pesticides. Because they had very different densities and for adsorption being dependent on carbon content, it was decided to introduce the same amount (100 mg) of carbon (implying different introduced masses) of each substrate in glass centrifuge tubes corresponding to 5.11 (SB), 3.98 (SA), 1.43 (SF), 0.25 (P) and 0.35 (F) g (dry weight). Triplicate samples of each substrate were prepared. Individual solutions of each pesticide were prepared in 0.01 M $CaCl_2$ at four solution concentrations 0.6, 1.7, 6.2 and 24.7 $\mu g\ L^{-1}$ by using both labelled and unlabelled

molecules. The 14C activity of the different solutions ranged between $6.42\times10^{-2}$, $5.42\times10^{-2}$ and $5.40\times10^{-2}$ MBq $L^{-1}$ for IPU, MTZ and EPX, respectively.

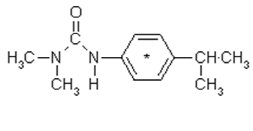
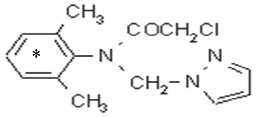
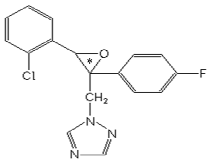
| Pesticide | Isoproturon | Metazachlor | Epoxiconazole |
|---|---|---|---|
| Abbreviation | IPU | MTZ | EPX |
| Action | Herbicide | Herbicide | Fungicide |
| Chemical family | Urea | Chloroacetamid | Triazole |
| Chemical formula | $C_{12}H_{18}N_2O$ | $C_{14}H_{16}ClN_3O$ | $C_{17}H_{13}ClFN_3O$ |
| Structural formula |  |  |  |
| Molecular weight (g $mol^{-1}$) | 206.28 | 277.75 | 329.76 |
| Water solubility (mg $L^{-1}$)[(a)] | 70.2 | 450.0 | 7.1 |
| Log($K_{ow}$) coefficient[(b)] | 2.5 | 2.5 | 3.3 |
| pka | No dissociation | No dissociation | No dissociation |
| Radio-labeled pesticide purities (%) | 94.7 | 93.2 | 97.9 |
| Specific activities (MBq $mmol^{-1}$) | 475 | 1636 | 1308 |
| Un-labeled pesticide purities (%) | 99.9 | 99.9 | 99.2 |

**Table II-2: Chemical characteristics of the pesticides. [(a)] 20 °C; [(b)] 20 °C, pH=7. The position of the labelling is indicated by the asterisk.**

According to preliminary kinetic experiments, equilibrium was considered to be reached after 24 hours. Tubes were rotated during 24 hours with an end-over-head shaker. After equilibrium, tubes were centrifuged at 1800 *g* for 15 min (Sorvall Evolution RC, Kendro, Courtaboeuf, France). Supernatants were removed and their radioactivities were measured in 1-mL aliquots added with 10 mL of Ultima Gold XR scintillation liquid (Perkin Elmer, Waltham, USA) using a Tri Carb 2100 TR liquid scintillation counter (Perkin Elmer Ins., Courtaboeuf, France). Control tests of molecule adsorption onto glassware or tube caps were carried out by reproducing the same procedures without substrate. 7, 4 and 8 % of initial radioactivity was adsorbed on the centrifuge tubes for MTZ, IPU and EPX, respectively. Sorbed amounts ($Qe_{ads}$ in mg $kg^{-1}$) were calculated from the difference between initial and equilibrium concentrations ($C_e$, $mg.L^{-1}$). The sorption data were described using the Freundlich equation:

$$Qe_{ads} = K_f C_e^{nf} \quad \text{(Eq. 8)}$$

where $K_f$ and $n_f$ are the Freundlich adsorption parameters, obtained after data ln-transformation. For $n_f$ coefficients close to 1 (between 0.95 and 1.05) (Calvet et al., 2005a), adsorption isotherms linearity allowed the equivalency between $K_f$ and the $K_d$ partition coefficient to be accepted:

$$K_d = Qe_{ads} / C_e \quad \text{(Eq. 9)}$$

Normalized adsorption coefficients were also determined by dividing $K_d$ values by the initial organic carbon content.

$$K_{oc} = K_d / f_{oc} \quad \text{(Eq. 10)}$$

where $f_{oc}$ is the organic carbon fraction (g $g^{-1}$).

Desorption experiments were conducted immediately after sorption experiments replacing 7 mL of supernatant with 0.01 M $CaCl_2$ pesticide-free solution. Suspensions were shaken for 24 h, centrifuged and the pesticide concentration measured as described above. Four successive desorption steps were carried out. Desorbed amounts were determined at

each sorption step and the remaining sorbed quantities ($Qe_{des}$) were described by Freundlich isotherms:

$$Qe_{des} = K_{fd} C_e^{nfd} \quad \text{(Eq. 11)}$$

where $K_{fd}$ and $n_{fd}$ are the Freunlich desorption parameters.

The desorption process sometimes requires a larger energy than that needed to adsorb molecules. This is translated by a more pronounced curvature of the desorption isotherms than that of the adsorption isotherms, which is usually described by a hysteresis coefficient (H) calculated as follows:

$$H = \frac{n_{fd}}{n_f} \times 100 \quad \text{(Eq. 12)}$$

Hystereris is generally apparent only for H < 70 % (Barriuso et al., 1994).

#### 4.3.4 Statistics

Statistical analyzes were conducted using the R software (R Development Core Team, 2005). The non-parametric Kruskal-Wallis test was first computed to detect statistically significant ($\alpha$ = 0.05) differences among the five substrates for $K_d$ and $K_{oc}$. The post hoc Steel-Dwass test (Day and Quinn, 1989) was subsequently performed for pair-wise comparisons for dataset presenting significant heterogeneity. A multi-linear regression (MLR) analysis was computed to link $K_d$ to clay content, organic carbon content, pH and CEC. Among these four parameters, those presenting no significant correlation were removed one-by-one from the MLR analysis.

### *4.4 Results and Discussion*

#### 4.4.1 Adsorption

For all substrates, adsorption isotherms were well described by Freundlich model (Table II-3) with $R^2$ larger than 0.995 for IPU and MTZ and larger than 0.984 for EPX.

##### *4.4.1.a Sorption coefficients*

The $n_f$ coefficient expresses the isotherm curvature. Observed $n_f$ ranged from 0.93 to 1.22 (Table II-3). Overall, apart from EPX, $n_f$ values did not largely depart from unity, thus allowing for the calculation of $K_d$ and $K_{oc}$ linear coefficients. However, slight differences in $n_f$ values can be seen among the three pesticides. MTZ $n_f$ were usually very close to unity [0.98 – 1.03], although they were not significantly different from those of IPU or EPX. Isotherm pseudo-linearity indicates that there is no concentration effect on MTZ adsorbed amounts. Allen and Walker (1987) reported $n_f$ coefficient values frequently lower than unity for MTZ sorption on 18 contrasted soils. EPX $n_f$ coefficients were significantly higher than those of IPU and slightly higher than unity. The three sediments and soil substrates (SB, SA and SF) had the highest EPX $n_f$ coefficients ranging from 1.08 to 1.22. Conversely, isotherm curvature was less pronounced for the vegetal substrates P and F. As reported by Calvet (1989), S-shaped isotherms ($n_f$ > 1) can be observed for the adsorption of organic molecules on clay surfaces. However, triazole adsorption isotherms were mostly described by L-shapes for molecules with high hydrophobicity and low solubility (Singh, 2002; Rodriguez-Cruz et al., 2006) and in few cases according to S-shaped isotherms (Singh, 2002). According to its log($K_{ow}$) (Table II-1), EPX is hydrophobic. However, the presence of two aromatic groups and F and Cl atoms implies that EPX is a complex molecule with a lower solubility (7.1 mg/L at 20°C) than that of the triazole pesticides studied by Singh (2002) (hexaconazole, triadimefon, penconazole) and Rodriguez-Cruz et al. (2006) (penconazole). These characteristics may explain EPX ease at being adsorbed on mineral or organic material.

Except for P ($n_f$=1.04), IPU $n_f$ coefficients were lower than unity (L-shaped isotherms) as reported by previous studies (Coquet, 2003; Boivin et al., 2005b). Forest litter $n_f$ value for IPU was close to unity. Wetland sediments, forest soil and leaves have a high affinity for IPU.

| Molecule | Substrate | Sorption | | | | | |
|---|---|---|---|---|---|---|---|
| | | $K_f$ | $K_{f/oc}$ | $n_f$ | $R^2$ | $K_d$ | $K_{oc}$ |
| | | $mg^{1-nf}\ kg^{-1}\ L^{nf}$ | | - | - | $L\ kg^{-1}$ | |
| MTZ | SB | 4 | 180 | 1.01 | 0.999 | 3.2 ± 0.2 | 166 ± 10 |
| | SA | 5 | 205 | 1.03 | 0.999 | 4.1 ± 0.4 | 255 ± 26 |
| | SF | 14 | 207 | 0.98 | 1.000 | 16.6 ± 0.9 | 240 ± 13 |
| | P | 60 | 148 | 1.02 | 1.000 | 53 ± 3 | 131 ± 6 |
| | F | 53 | 186 | 1.01 | 0.997 | 49 ± 5 | 176 ± 18 |
| IPU | SB | 4 | 219 | 0.93 | 1.000 | 7 ± 1 | 372 ± 63 |
| | SA | 3 | 134 | 0.95 | 0.999 | 4.7 ± 0.5 | 292 ± 33 |
| | SF | 14 | 203 | 0.93 | 0.999 | 24 ± 3 | 342 ± 48 |
| | P | 44 | 108 | 1.04 | 0.995 | 34 ± 6 | 84 ± 15 |
| | F | 41 | 144 | 0.99 | 0.999 | 43 ± 4 | 152 ± 13 |
| EPX | SB | 155 | 7906 | 1.08 | 0.996 | 76 ± 17 | 3939 ± 851 |
| | SA | 408 | 16240 | 1.19 | 0.987 | (a) | (a) |
| | SF | 2489 | 35624 | 1.22 | 0.984 | (a) | (a) |
| | P | 902 | 2218 | 1.06 | 0.993 | 546 ± 136 | 1356 ± 338 |
| | F | 535 | 1885 | 1.01 | 0.999 | 546 ± 151 | 1950 ± 539 |

**Table II-3: Adsorption Freundlich and linear parameters for metazachlor (MTZ), isoproturon (IPU) and epoxiconazole (EPX) on Bray wetland sediments (SB), Aulnoy wetland sediments (SA), forest soil (SF), Bray wetland plants (P) and forest litter (F). (a) Unreliable $K_d$ and $K_{oc}$ values due to $n_f$ values outside the boundary of linearity assumption acceptability ($0.95 < n_f < 1.05$). (b) Not calculated for large $n_f$ departure from unity.**

Benoit et al. (2008) also found L-shaped isotherms for IPU sorption on forest litter. As a consequence, one can expect decreased accessibility to sorption sites when liquid phase concentration increases (Calvet, 1989). However, it should be noted that on-site pesticide concentrations at the inlet of the buffer zones rarely exceed 5 µg $L^{-1}$. Consequently, there are few chances that pesticide sorption process be limited by site accessibility.

$K_f$ sorption coefficients provide an indication of the sorption capacity of a substrate for a specific molecule. This parameter corresponds to the isotherm slope. $K_f$ values were normalized to the substrate carbon content ($K_{f/oc}$). $K_{f/oc}$ values varied widely from 148 to 207 (MTZ), 108 to 219 (IPU) and 1885 to 35624 (EPX) $mg^{1-n_f}\ kg^{-1} L^{n_f}$. Comparing pesticide $K_{f/oc}$ values should be done cautiously because their units depend on $n_{fa}$ values. Linear $K_{ocs}$ were therefore preferentially discussed when linearity assumption was fulfilled (Table II-3).

*4.4.1.b Wetland sediments (SB and SA) and forest buffer soil (SF) sorption capacities*

On wetland sediments and forest soil, IPU sorption parameters ($K_f$, $K_d$ and $K_{oc}$) were generally larger than or similar to those of MTZ (Table II-3). IPU and MTZ had similar moderate log($K_{ow}$) coefficients (FOOTPRINT, 2010). However, MTZ solubility was higher than that of IPU which may explain the slightly higher affinity of wetland sediments and forest soil for IPU than MTZ. For MTZ, SB had a significantly lower $K_{oc}$ than SA. For IPU, all sorption coefficients on SB were higher than on SA (Table II-3). IPU and MTZ may

present different behaviours in terms of adsorption. SA composition included less clay and organic matter contents but more $CaCO_3$ than that of SB. SA pH was also more basic than that of SB. For both IPU and MTZ, the forest soil adsorption coefficients were equal to or larger than those calculated on the wetland sediments. Overall, the $K_{oc}$ values for IPU and MTZ were in the upper range of the literature values, or even larger than previously reported values from soil studies (FOOTPRINT, 2010). It seems that the more clayey SB wetland sediments and SF forest soil were more favourable for IPU sorption than SA sediments. MTZ was less affected by sediments or soil types than IPU was.

Very large $K_f$ and $K_{f/oc}$ values were reported for EPX on these three substrates which did not exhibit significant differences among them. Forest soil $K_{f/oc}$ value for EPX was approximately two- and five-fold higher than that of SA and SB, respectively. These values are larger than those reported in the literature (Roy et al., 2000; FOOTPRINT, 2010). The use of $K_{oc}$ values from soil studies to model pesticide adsorption on buffer zones substrates is therefore not recommended.

For SF, $n_f$ values are low for IPU and high for EPX. Comparisons based on sorption coefficients are therefore difficult. However, it appeared that, on SF, all sorption coefficients were lower for MTZ and IPU than for EPX. The same tendency was observed for SB and SA sediments as well. The hydrophobicity of the molecule explained the greater sorption of EPX compared to the moderately mobile IPU or MTZ herbicides on soil or sediments components. In a previous study, Benoit et al. (2008) found that an oak/chesnut forest soil characterized by high particulate organic matter hydrophobicity had a high sorption capacity for IPU (moderately mobile) and diflufenican (hydrophobic). Given the extremely high $K_{f/oc}$ value of EPX, the forest soil from the Bray catchment demonstrated a very high potential for EPX sorption as also found for IPU and MTZ.

*4.4.1.c Wetland plants (P) and forest litter (F)*

MTZ sorption was higher than that of IPU on wetland plants and forest leaves as attested by $K_f$, $K_d$ and $K_{oc}$ results (Table II-3). However, these differences were only significant on plants. It therefore seems that MTZ sorption is more affected than IPU by the presence of fresh vegetal sorbents such as wetland plants forest litter. As found on all substrates, EPX sorption on P and F was significantly greater than that of the other two molecules. However, no significant difference between P and F could be found indicating that EPX sorption is apparently not affected by substrate quality. Margoum et al. (2005) noted that for agricultural ditches, sorption of different herbicides was larger on dead leaves than on bottom sediments. Our results suggest that organic matter from wetland plants seemed to have lower affinity for pesticide adsorption than forest leaves. However, this should not lessen the fact that, even though it had the lowest $K_{f/oc}$, plants average $K_{f/oc}$ was not negligible, compared to that of the other substrates. The presence of vegetation in wetlands can therefore play a significant role for the retention of moderately mobile molecules like IPU or MTZ. Wechsler et al. (1996) showed that the fresher the organic matter (the higher the C/N ratio), the higher the adsorption of two triazole fungicides (flutriafol and flusilazol). Highly hydrophobic molecules like EPX would not require as much organic matter or clay content for retention than more mobile molecules would.

Multi-linear regressions were fitted to $K_d$ data (n=36) obtained on the two sediments and the forest soil to detect possible dependency of this adsorption parameter to four main characteristics of these three substrates: clay content, organic carbon content, pH and CEC (Table II-4).

| Molecule | intercept | Clay | OC | $R^2$ |
|---|---|---|---|---|
| | | $g\ kg^{-1}$ | $g\ kg^{-1}$ | |
| Metazachlor | 2.65 | -0.01 | 0.25 | 0.99 |
| Isoproturon | -2.96 | 0.009 | 0.35 | 0.94 |
| Epoxiconazole | 18.93 | -0.11 | 5.01 | 0.79 |

**Table II-4: Multi-linear regression models (*n*=36) for $K_d$ for the three pesticides, derived from data obtained on SB, SA and SF. Clay content, organic carbon content, CEC and pH were tested for the best model fit.**

For the three molecules, neither pH nor CEC could be included into regression models showing a lower importance of these parameters for IPU, MTZ and EPX sorption on such substrates. The final models showed fairly high correlation coefficients (>0.79). Organic carbon seemed of primary importance but clay content also had a significant, but lower influence on pesticides $K_d$. In addition, increase in clay content seemed to enhance IPU and EPX sorption but decrease that of MTZ. For geological substrates with very poor organic matter content, Coquet et al. (2004) observed good correlation between IPU $K_d$ and clay content, contrary to Weber et al. (2004) who included a wider variety of soils. Clay content seemed to exert a smaller influence on MTZ adsorption than the organic carbon content as also noted by Rouchaud et al. (1996). Organic carbon content (Singh, 2002) and nature (Wechsler et al., 1996) as well as clay content (Singh, 2002) were shown to affect triazole compounds sorption, whereas no influence of these parameters was found according to the EPX European report (European Commission, 2006).

### 4.4.2 Desorption

Desorption isotherms were well fitted by Freundlich models, particularly for the moderately mobile herbicides. Models $R_d^2$ varied from 0.949 to 0.997 (MTZ), 0.780 to 0.999 (IPU) and 0.468 to 0.985 (EPX) (Table II-5).

| Molecule | Substrate | Desorption parameters (from 20 µg $L^{-1}$) | | | |
|---|---|---|---|---|---|
| | | $K_{fd}$ | $n_{fd}$ | $R^2_d$ | H |
| | | $mg^{1-nfa}\ kg^{-1}\ L^{nfa}$ | - | - | % |
| MTZ | SB | 0.11 | 0.31 | 0.985 | 30.2 |
| | SA | 0.11 | 0.28 | 0.969 | 26.7 |
| | SF | 0.69 | 0.39 | 0.949 | 39.3 |
| | P | 3.35 | 0.42 | 0.996 | 41.5 |
| | F | 2.49 | 0.39 | 0.997 | 38.5 |
| IPU | SB | 0.10 | 0.20 | 0.999 | 19.7 |
| | SA | 0.10 | 0.24 | 0.913 | 22.8 |
| | SF | 0.25 | 0.14 | 0.780 | 14.6 |
| | P | 26.56 | 0.94 | 1.000 | 91.9 |
| | F | 4.51 | 0.53 | 0.963 | 52.4 |
| EPX | SB | 0.09 | 0.07 | 0.468 | 6.7 |
| | SA | *0.02* | *-0.15* | *0.650* | *-14.1* |
| | SF | 0.30 | 0.05 | 0.733 | 5.3 |
| | P | 2.34 | 0.12 | 0.985 | 11.5 |
| | F | 1.14 | 0.06 | 0.880 | 5.6 |

**Table II-5: Desorption characteristics on metazachlor (MTZ), isoproturon (IPU) and epoxiconazole (EPX) from Bray wetland sediments (SB) and plants (P), Bray forest soil (SF) and litter (F) and Aulnoy wetland sediments (SA). Data from initial concentrations of 20 µg $L^{-1}$.**

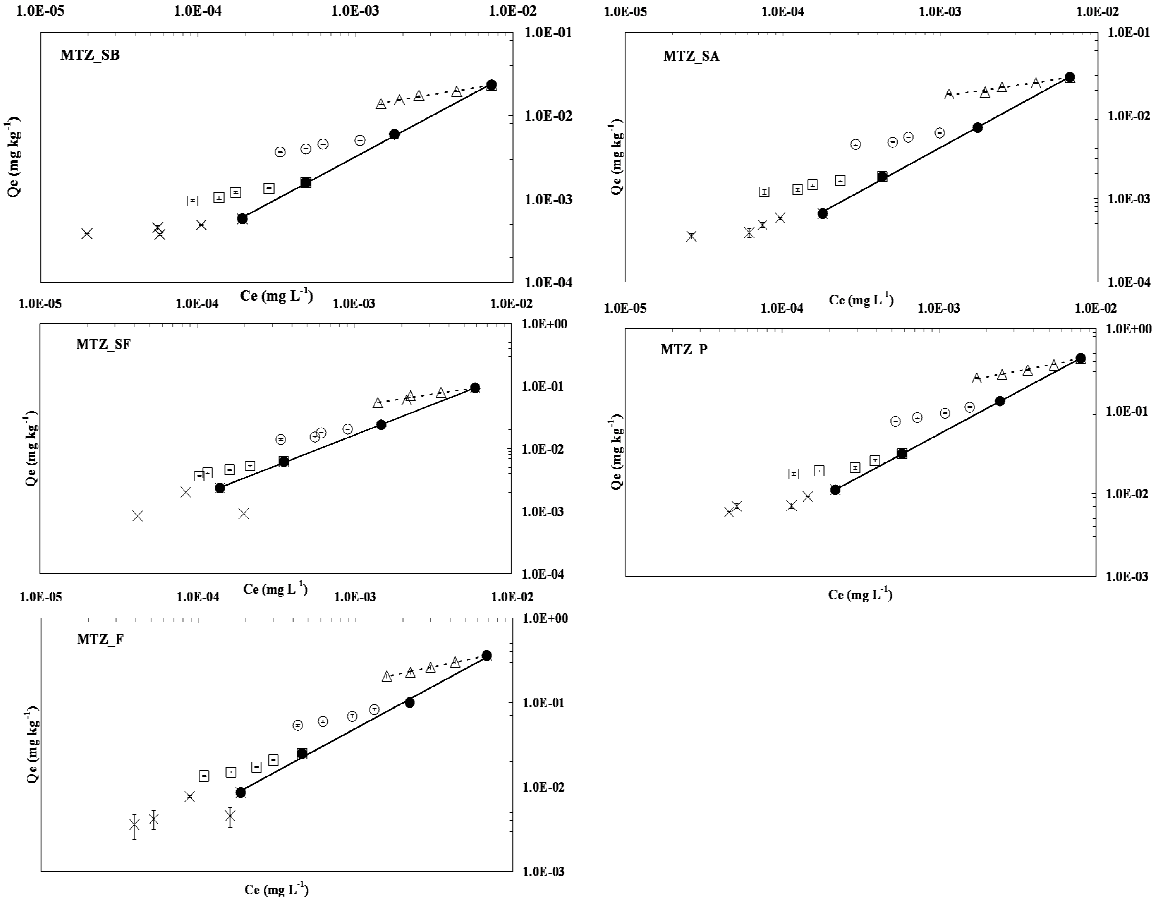



**Fig. II-1: Adsorption (full symbols) and desorption (empty symbols) isotherms of metazachlor (MTZ) on the Bray (SB) and Aulnoy (SA) wetland sediments, forest soil (SF), wetland plants (P) and forest litter leaves (F). Desorption isotherms from 20 (△), 5 (○), 1.34 (□) and 0.5 (×) µg/L were initiated from adsorption points. Black lines represent Freundlich sorption isotherms.**

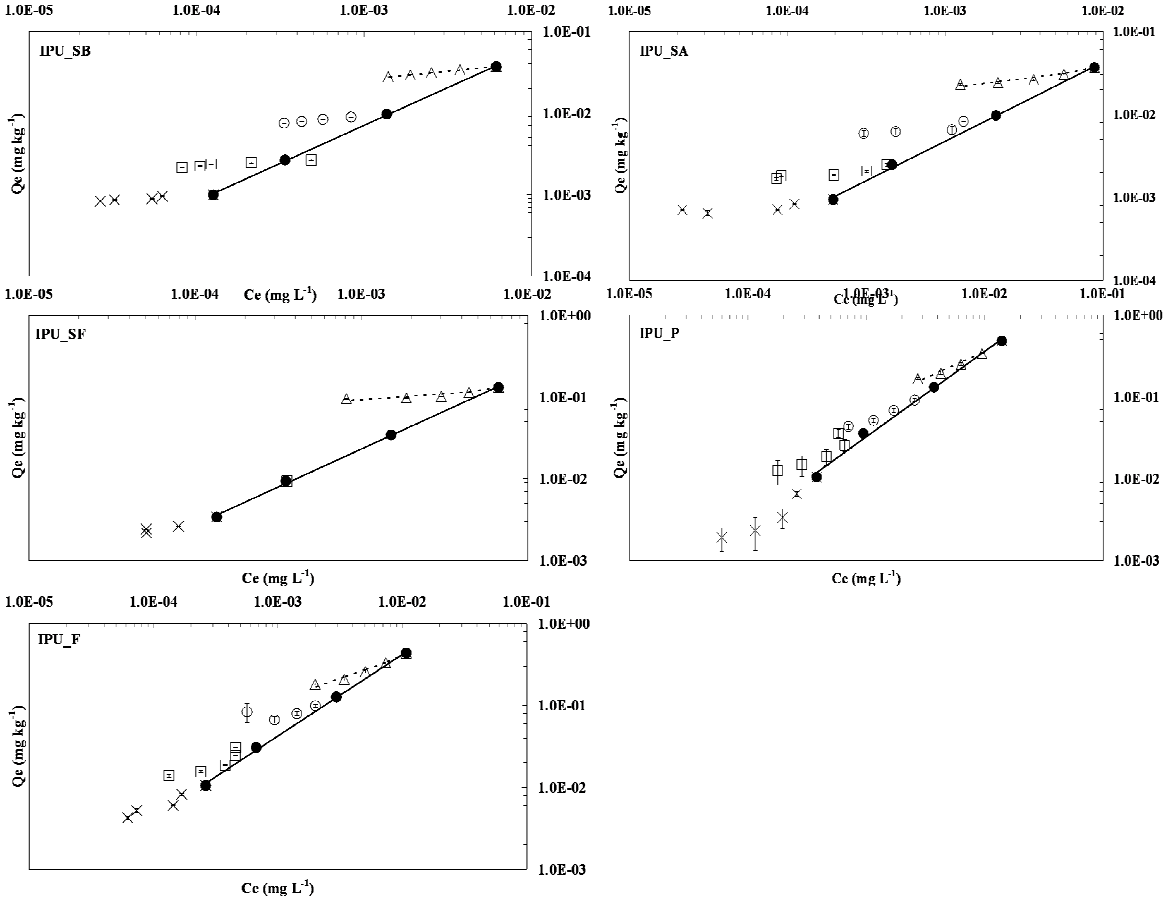



**Fig. II-2: Adsorption (full symbols) and desorption (empty symbols) isotherms of isoproturon (IPU) on the Bray (SB) and Aulnoy (SA) wetland sediments, forest soil (SF), wetland plants (P) and forest litter leaves (F). Desorption isotherms from 20 (△), 5 (○), 1.34 (□) and 0.5 (×) µg/L were initiated from adsorption points. Black lines represent Freundlich sorption isotherms.**

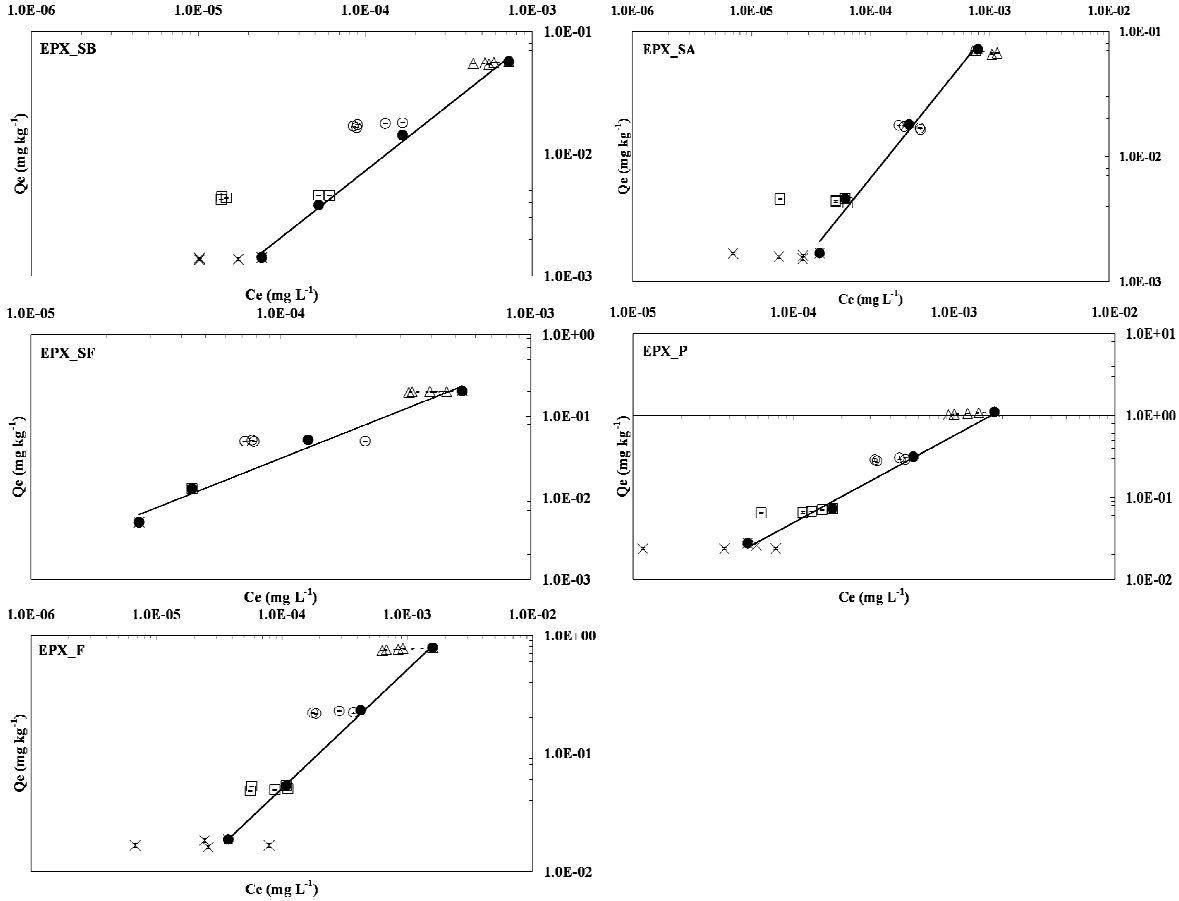



**Fig. II-3: Adsorption (full symbols) and desorption (empty symbols) isotherms of epoxiconazole (EPX) on the Bray (SB) and Aulnoy (SA) wetland sediments, forest soil (SF), wetland plants (P) and forest litter leaves (F). Desorption isotherms from 20 (△), 5 (○), 1.34 (□) and 0.5 (×) µg/L were initiated from adsorption points. Black lines represent Freundlich sorption isotherms.**

### *4.4.2.a Moderately reversible adsorption: IPU and MTZ*

For the two wetland sediments as well as the forest soil, IPU $n_{fd}$ were lower than MTZ $n_{fd}$ all values being lower than 0.40. A moderate hysteresis was therefore apparent (Fig. II-1 and Fig. II-2) associated to hysteresis coefficients (H) pertaining to the [14.6–22.8] (IPU) and [26.7–39.3] (MTZ) % ranges (Table II-5). Very similar shapes were observed for IPU and MTZ for the wetland sediments, whereas MTZ desorption isotherms looked S-shaped for the forest soil (Fig. II-1). Hysteresis indicates that the energy needed to desorb previously adsorbed molecules was greater than that needed to adsorb them. Only a portion of previously adsorbed IPU and MTZ was desorbed. Clay content was shown to exert a significant positive influence on IPU adsorption but to a lower extent than organic carbon content. Conversely, as found for the adsorption process, MTZ seemed less affected by sediment composition than IPU. Indeed, no significant difference was detected between SB and SA for MTZ which presented the same $K_{fd}$ values. Compared to the results presented in this study, Mamy and Barriuso (2007) found similar hysteresis coefficients (from 18 to 39 %). These authors also highlighted that $K_{fd}$ depend on previously adsorbed amount. Consequently, comparing $K_{fd}$ among substrates should be done cautiously. Overall, on these three substrates, IPU hysteresis and Freundlich desorption coefficients were lower than those of MTZ. It therefore seems that IPU was more resistant to desorption than MTZ which may be explained by MTZ higher solubility. Contrary to what has been observed for the wetland sediments and the forest soil, IPU desorption parameters ($K_{fd}$, $n_{fd}$ and H) were higher on wetland plants and forest leaves than those of MTZ (Table II-5) but differences were not significant. Adsorption of both IPU

and MTZ on the two vegetal substrates was reversible although it seemed that IPU was more easily desorbed than MTZ. IPU desorption isotherms were close to adsorption isotherms for both plants (H=64.1 %) and leaves (H=52.4 %) (Fig. II-1 and Fig. II-2). MTZ desorption from P and F seemed slightly more pronounced than that observed on wetland sediments and forest soil. It supports the fact that the adsorption of these two moderately mobile herbicides on such organic materials was reversible and occurred to a greater extent for IPU.

#### *4.4.2.b Weakly reversible adsorption: EPX*

For the five substrates, all desorption parameters for EPX were much lower than those estimated for IPU and MTZ. On SA sediments, desorbed EPX varied widely from one desorption step to another thus leading to aberrant values and negative $n_{fd}$. On all substrates, liquid phase counted radioactivity was very low (only between two to five times larger than the radioactivity background noise). For such low radioactivity counts, it is delicate to conclude on EPX evolution throughout the successive desorption steps. Apart from SA, the other four substrates exhibited very hysteretic desorption isotherms (Fig. II-3) with H values ranging from 5.6 to 11.5 % (Table II-5) indicating low desorption of EPX. EPX sorption was almost not reversible, particularly on sediments and forest soil.

### *4.5 Conclusions*

These laboratory experiments clearly demonstrated a high potential of wetland or forest main substrates for pesticide retention. From a water quality perspective, irreversible adsorption is a first step that could be targeted as it removes pesticides from water. Further, reversible adsorption is not necessarily harmful as it helps attenuating concentration peaks by delaying pesticide transfer. Sorption hysteresis influences convection and dispersion processes by delaying pesticide elution which will be slowed down. In wetlands or forest buffers, sediments or soil seemed to play the most important role in pesticide sorption, compared to wetland vegetation or forest litter. However, vegetal substrates exhibited a high sorption potential but also had the highest desorption rates. Sorption interactions on such organic material are therefore suspected to be of weak energy. Metazachlor sorption was more affected by plants (less reversible adsorption) than isoproturon. For epoxiconazole, almost no difference could be detected among substrates, which all showed high and almost irreversible sorption potentials. It seems that hydrophobic molecules such as epoxiconazole are less demanding for adsorption in terms of organic matter and clay content or quality. Buffer zones may behave as permanent sinks into which such hydrophobic molecules may accumulate. This should be taken into consideration in designing wetland and further degradation of pesticides like EPX should be carefully studied. Despite presenting similar low hydrophobicity, metazachlor and isoproturon showed different behaviours that may be explained by metazachlor higher solubility. In addition, it was noted that the more clayey Bray wetland sediments and forest soil had a higher affinity for isoproturon than metazachlor leading to less reversible sorption. The forest soil usually showed a higher sorption potential than the wetland sediments thus demonstrating promising results for the use of forest as buffer for pesticide pollution. In addition, the forest litter could also impact pesticide transfer, at least temporarily, as attested by their high sorption, but non-negligible desorption, potentials. However, it seemed that the forest soil, fairly clayey, provided a higher sorption capacity than the leaves themselves. Practically, it can be advised that introducing plants in wetlands, or leaving accumulated litter in forests or vegetated ditches, may provide additional pesticide pollution reduction through adsorption processes.

*4.6 Acknowledgements*

This research benefited from the FIRE (Fédération Ile-de-France de recherche sur l'environnement) and ArtWET LIFE (06/ENV/F/000133) financial supports. The authors also want to thank V. Dumeny for helpful laboratory work contribution.

## 5 Artificial wetland and forest buffer potential for epoxiconazole degradation

*This part is in preparation and will be submitted to Chemosphere.*

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*5.1 Abstract*

Epoxiconazole, a broad-spectrum fungicide, can be transported by subsurface tile drains and contaminate connected surface waters. In response to rising concern over agricultural pesticide losses to the environment, current research is investigating the utility of buffer zones like artificial wetlands and forest buffers to minimize pesticide effects on surface waters. Pesticide fate in such systems has not been extensively characterized. This study was focused on the potential of degradation of epoxiconazole in several substrates under flooded (anoxic) conditions: wetland sediments, wetland plants, forest soil and litter taken at two sites in France. $^{14}C$ radio-labelled epoxiconazole mineralization was slow and low (< 4 % in 177 days) but unidentified metabolites were produced. Apart from Bray wetland sediments, a lag-phase was observed and maximal mineralization rates were not reached after 177 days of incubation. Water extractable fraction of radioactivity did not exceed 8 % along the incubation period except for wetland plants (18.8 % at day 177), whereas methanol extracts decreased on average from 100 to 76 %. Non-extractable residues (NER) increased up to approximately 18 % except for wetland plants which were associated with larger fractions of NER (29.8 %) at the end of the incubation period. Plants fresh organic matter seemed to enhance epoxiconazole removal through NER formation. A modified kinetic lag-phase model was proposed. Estimated disparition half-lives on wetland sediments were lower than 65 days but those from forest soil, wetland plants and forest litter were higher (339 – 371 days). The importance of vegetation as support for microbial growth and NER formation was noticed. Reducing conditions did not appear to be favourable for epoxiconazole mineralization although degradation and NER formation occurred. In field conditions, this could be compensated by the temporary aerobic conditions created by water level fluctuations.

**Keywords**: Wetlands, forest, epoxiconazole, pesticide, pollution, degradation

*5.2 Introduction*

Epoxiconazole is a fungicide widely used on wheat, sugarbeet, triticale, barley and oat (Tomlin, 2003). Despite its low solubility and high hydrophobicity, it has been frequently detected at the outlet of artificially drained catchments (Passeport et al., 2010a). The European Water Framework Directive 2000/60/EC requires a good status of water bodies to be reached by 2015. In complement to pesticide application reduction, artificial wetlands and forest buffers are being studied for their potential to improve water quality. However, such buffer zones' functioning regarding pesticide pollution is still unclear. Wetland sediments and plants as well as forest soil and litter could act as possible substrates onto which microbial populations may develop and further degrade contaminants. The specific effect of these substrates has not been extensively characterized. Particularly, little is known about epoxiconazole fate in aquatic ecosystems. Most information on its persistance is derived from registration documents and deals with aerobic studies conducted on agricultural soils (European Commission, 2006). Epoxiconazole degradation is slow under aerobic conditions (Montfort et al., 1997; Bromilow et al., 1999a) with field studies half-lives ranging from 44 to 226 days (FOOTPRINT, 2010). Literature concerning its fate under flooded conditions is scarce (Lin et al., 2001; Buerge et al., 2006; European Commission, 2006). Under flooded conditions, pesticides can undergo reductive transformations like dehalogenation which is generally the initial metabolic step under anoxic conditions (Wolfe, 1992; Häggblom et al., 2000). Differing results were found on the effect of reductive conditions on pesticide degradation depending on molecules. It was suggested that the epoxiconazole oxirane-ring could cleave to form BF-480 entriazol alkene. This metabolite accounted for 5.7 and 32.7 % of applied radioactivity after 100 days and 8.6 % after 120 days for different soils or sediments under flooded conditions (Buerge et al., 2006; European Commission, 2006). A study was carried out on paddy rice soil mesocosms where epoxiconazole half-lives ranged between 20 and more than 97 days (Lin et al., 2001). This suggests that results concerning epoxiconazole fate under reducing conditions are highly variable. A better understanding of wetlands and forest buffers functioning to control pesticide pollution is needed to help design and optimize these systems. The objective of this study was to test wetland sediments, wetland plants, forest soil and forest litter for their potential to dissipate epoxiconazole under controlled laboratory flooded conditions. The use of radio-labelled epoxiconazole allowed complete mass balance calculations. Disparition and mineralization first-order kinetic models were tested for their ability to simulate epoxiconazole fate data for different buffer zone substrates.

## *5.3 Materials and methods*

### 5.3.1 Substrates

A forest buffer and an artificial wetland located at Bray (Indre-et-Loire, France, 47°03'N, 01°17'E) (Passeport et al., 2010b) were sampled for wetland sediments (0 – 10 cm, SB) and plants (P) and forest soil (0 – 10 cm, SF) and litter (F). Sediments (0 – 10 cm, SA) were also taken at a second wetland located at Aulnoy (Seine-et-Marne, France, 48°50'N, 03°06'E). The three buffer zones receive water from small artificially drained agricultural catchments (< 50 ha). Wetland plants were mixed residues of *Glyceria Maxima* (71 %), *Festuca Arundinacea* (16 %) and *Phragmites Australis* (13 %), whereas forest litter was made of dead oak tree leaves. Wetland sediments (SB and SA) and forest soil (SF) were sieved to 2 mm. Plants and leaves were crushed and sieved to 5 mm. The Bray sediments (SB) and forest soil (SF) were silty clay loams and had 1.94 and 6.91 % organic carbon (OC) content, and pH values of 7.38 and 6.75, respectively. The Aulnoy sediments (SA) was classified as a loam with 1.62 % OC and a slightly basic pH (8.1) (Table II-1).

### 5.3.2 Pesticide

$^{14}$C-oxirane-labelled epoxiconazole (2*RS*,3*SR*)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1*H*-1,2,4-triazole (EPX) was purchased from Izotop (Budapest, Hungary). Its purity was 97.9 % and its specific activity was 1308 MBq mmol$^{-1}$. Considering its low water solubility (7.1 mg L$^{-1}$) and high log($K_{ow}$) coefficient (3.3), EPX can be considered a hydrophobic fungicide (FOOTPRINT, 2010).

### 5.3.3 Incubation experiments

$^{14}$C-EPX disparition was monitored through incubation experiments. Substrates (1 g of initial carbon) were incubated in the dark at approximately 27 ± 2 °C in sealed 500 mL jars with unfiltered water (80 g) sampled at the inlets of the Bray and Aulnoy buffer zones. Initial dry weights were 51.1 (SB), 39.8 (SA), 14.3 (SF), 2.5 (P) and 3.5 g (F). Vials containing NaOH (2 mL, 3N) were introduced in jars to trap released $CO_2$. Substrates were pre-incubated during 48 hours before $^{14}$C-EPX solution was introduced with a targeted initial concentration of 25 µg/L and an 8.52 MBq L$^{-1}$ initial radioactivity. $^{14}$C-EPX disparition was monitored for 177 days. NaOH vials were changed every two weeks and 10-mL Ultima Gold XR scintillation liquid (Perkin Elmer, Waltham, USA) was added. Trapped $^{14}$C-$CO_2$ was measured using a Tri Carb 2100 TR liquid scintillation counter (Perkin Elmer Ins., Courtaboeuf, France) for approximately 24 hours after scintillation liquid addition to prevent bias induced by chemiluminescence.

At four time steps (0, 14, 77, and 164 days after $^{14}$C-EPX addition), samples were transferred to centrifuge tubes with 10 mL 0.09 M $CaCl_2$. Tubes were shaken with an end-over-head agitator for 24 hours at room temperature and centrifuged (6000 g, 10 min). Supernatant was removed and radioactivity was measured to estimate water extractable fraction. Thereafter, three additional extractions were successively carried out using 70 mL methanol (CarloErba, Val de Reuil, France). Triplicate samples were sacrificed for each substrate and time step. After extraction, substrate samples containing non-extractable $^{14}$C-EPX residues (NER) were air-dried (30 °C). Sediment and soil substrates were manually crushed whereas plants and leaves were crushed by a Retsch MM400 ball mill (Haan, Germany). Their radioactivity content was measured after combustion in an Oxidizer OX700 (Zinsser-Analytic GMBH, Frankfurt, Germany) at 900 °C under nitrogen flow (Air Liquide, Limay, France) and trapping of $^{14}$C-$CO_2$ with Oxysolve C-400 cocktail (Zinsser-Analytic, Frankfurt, Germany) prior to liquid scintillation counting.

One additional incubation system was set up for each substrate without pesticide. Only substrates and water were added in the same amount as those of the $^{14}$C-EPX incubation experiments. Microbial biomass activity was deduced from these EPX-free systems by monitoring trapped $CO_2$ measured by colorimetric continuous flow analysis (SA-40 analyser, Skalar, Breda, the Netherlands). Flooded substrates ensured low dissolved oxygen content whose concentration was regularly monitored by an OxiCal-SL CellOx 325 probe (WTW, Weilheim, Germany). Gas phase was analyzed at day 160 by a gas chromatography (micro-gc CP-4900 Quad, Varian, and CPMAITRE ELITE software 3.2, Les Ulis, France).

### 5.3.4 Chemical analysis by HPLC

$CaCl_2$ and methanol extracts were concentrated and analyzed by High Performance Liquid Chromatography (HPLC) to identify whether radioactivity could be attributed to EPX or other unidentified molecules. $CaCl_2$ extracts were filtrated (Fisher Bioblock 90 mm glass microfibre filter discs) and concentrated through solid-phase extraction (SPE) cartridges (C18 cartridges Isolute ENV+, IST, Hengoed, UK) using 6 mL acetonitrile elutant (CarloErba, Val de Reuil, France). Cartridges were previously conditioned by passing through them three times 2 mL acetonitrile and three times 2 mL MilliQ water. The average recovery for this SPE concentration step was 92.16 ± 2.06 %. The three methanol extracts were combined and evaporated through an R-200 Rotavapor (Büchi, Flawil, Switzerland) under vacuum conditions and a temperature of 40 °C. Concentrated SPE elutes and residual solutions from methanol evaporation were then centrifuged 13400 tour/min (miniSpin, Eppendorf, Hamburg, Germany). Supernatants were analyzed by HPLC (600E Multisolvent Delivery System 717 Autosampler Waters, Milfort, MA, USA) and detection used a radioactivity flow detector (Packard-Radiomatic Flo-One Beta A500, Perkin Elmer, Waltham, USA). HPLC used a Nova-Pak C18 column (Waters, 250 mm x 4.6 mm ID, 4-µm particle size, 60 Å pore size). The HPLC mobile phase gradient increased progressively from 45/55 (v/v) methanol/water to 100 % methanol at a 1 mL min$^{-1}$ flow during 40 min. Injected volumes ranged from 100 to 300 µL according to estimated radioactivity of the samples. Under these conditions, EPX retention time was 24.5 min.

5.3.5 Data analyzes

Mineralization kinetics is generally fitted to a first-order model:

$$C_{CO_2}(t) = C_{CO_2\max} \times \left[1 - \exp\left(-k_{CO_2} \times t\right)\right] \quad \text{(Eq. 13)}$$

where $C_{CO_2}(t)$ and $C_{CO_2\max}$ are $CO_2$ concentrations (µg $L^{-1}$) at time t and for large values of time and $k_{CO_2}$ is kinetic constant ($d^{-1}$). In the case of the presence of a lag-phase, a modified first-order model was proposed and fitted allowing for the kinetic constant to be time-dependent:

$$C_{CO_2}(t) = C_{CO_2\max} \times \left[1 - \exp\left(-k_{CO_2\max}\left(1 - \exp\left(-\frac{t}{\tau_{CO_2}}\right)\right) \times t\right)\right] \quad \text{(Eq. 14)}$$

where $k_{CO_2\max}$ and $\tau_{CO_2}$ were maximal value of the kinetic constant and lag-phase, respectively.

In addition to mineralization, epoxiconazole disparition may also be due to degradation or NER formation. For disparition kinetics, a first-order kinetic model was tested on EPX concentrations:

$$C(t) = C_o \times e^{-k_{disp} \times t} \quad \text{(Eq. 15)}$$

where *C(t)* is EPX concentration measured at time t, $C_o$ is the initial EPX concentration and $k_{disp}$ is the disparition rate. For disparition kinetics presenting lag-phases, similarly to mineralization, the proposed tested model was:

$$C(t) = C_o \times \left[\exp\left(-k_{disp\max}\left(1 - \exp\left(-\frac{t}{\tau_{disp}}\right)\right) \times t\right)\right] \quad \text{(Eq. 16)}$$

where $C_o$, $k_{disp\max}$ and $\tau_{disp}$ were initial EPX concentration (µg $L^{-1}$), disparition kinetic constant ($d^{-1}$) and disparition lag-phase (d).

To avoid over-estimating half-lives derived from kinetic constants, the R software (R Development Core Team, 2005) was used to fit the models on untransformed data (Beulke and Brown, 2001). The non-linear least squares iterative method based on the Gauss-Newton algorithm was used. When the simultaneous adjustment of the three parameters was not possible, $\tau_{CO_2}$ (mineralization) or $\tau_{disp}$ (disparition) were manually adjusted and only $C_{CO_2\max}$ and $k_{CO_2\max}$ (mineralization) or $C_o$ and $k_{disp\max}$ (disparition) were derived from R. Occasionally, only manual fits were possible testing several values for kinetic parameters until the best fit was obtained. The selection between the classic first-order and modified lag-phase models was based upon comparisons between their residues sums of squares ($\sum e_i^2$). EPX mineralization and disparition half-lives were calculated using R software uniroot function and adding the lag-phase. The Tukey test was used (Einot and Gabriel, 1975) to detect which substrates presented significantly different percentages of mineralization.

*5.4 Results and Discussion*

5.4.1 Oxydo-reduction conditions

Dissolved oxygen concentrations rapidly decreased from the start of the experiment. Apart from few exceptions, all values were lower than 1 $mgO_2$ $L^{-1}$. It thus indicated that the targeted reducing conditions were met in the incubation systems. Because it was necessary to open and handle the jars regularly to either change NaOH vials or weight the systems, some introduction of oxygen occasionally occurred. However, oxygen diffusion in water is low (0.00197 $mm^2$ $s^{-1}$, 20°C) and it is unlikely that aerobic conditions remained over long periods. The measured overlaying gas phase composition was very similar to air composition but also included traces of methane production (Appendix V). Initial carbon conversion to methane after 160 days of incubation without pesticide was estimated to represent less than 25 %. Flooded conditions therefore helped maintaining targeted anoxic conditions.

5.4.2 Mineralization

Incubations including on-site water and substrates only, showed a significantly higher mineralization of SB organic carbon than that of the other substrates (Fig. II-4). In addition, no lag-phase seemed apparent for any of the substrates. A fairly steady state of $CO_2$ production was reached for SF, P and F after 42 days but $CO_2$ production was still increasing for SB and SA after 177 days. The microbial activity in the overlaying water was not significant compared to that of the substrates themselves. The wetland sediments, and particularly SB, therefore appeared to present higher microbial activity compared to the other treatments.

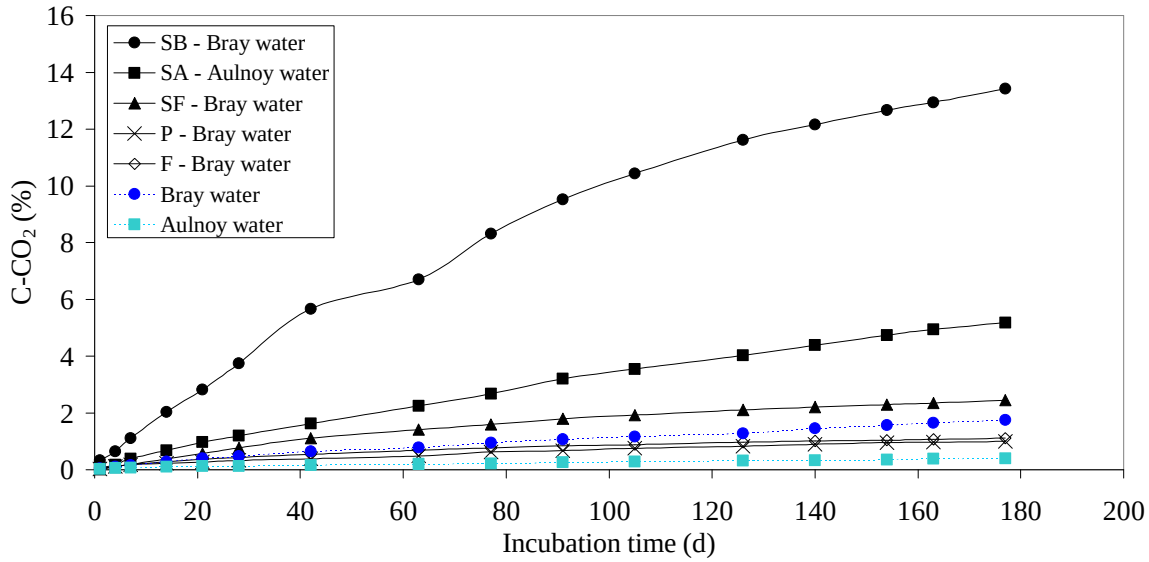


**Fig. II-4: C-$CO_2$ production from substrates or overlaying water in the absence of EPX (expressed as a percentage of the initial carbon content of each substrate). "SB - Bray water" means that is presented "SB" carbon mineralization "dismished by" that from "Bray water" column itself.**

$^{14}$C-EPX maximal mineralization has not been reached and is still slowly increasing at the end of the incubations (Fig. II-5). It therefore seems that after 177 days of incubation, $^{14}$C-EPX mineralization is still in a lag phase. The absence of lag phase for SB may be explained by its high microbial activity found in incubations without EPX (Fig. II-4).

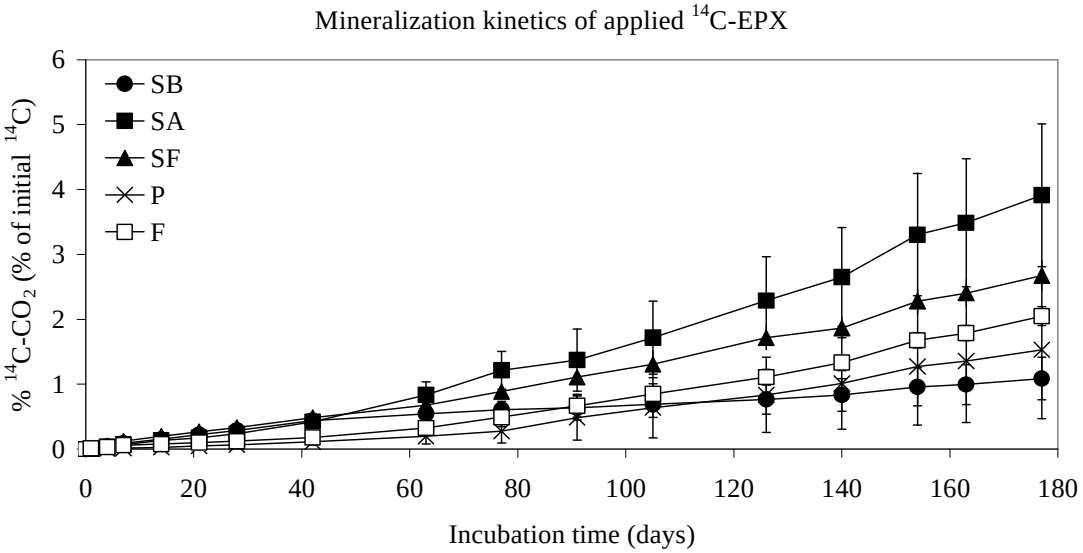


**Fig. II-5: Mineralization kinetics of applied $^{14}$C-EPX.**

As reported by the European EPX draft assessment report (European Commission, 2006), EPX hydrolysis is negligible under neutral and alkaline conditions, as were the wetland sediments and forest soil. All substrates considered, mineralization was lower than 4 % of initially introduced oxirane-labelled EPX after a 177-day incubation period. The two wetland sediments presented the most extreme results regarding EPX mineralization. At the end of the experiment, SB presented the lowest value (1.1 %), whereas SA showed the highest mineralization rate (3.9 %). SB percentage of mineralization was statistically different ($\alpha$=0.05) from that of SA, SF and F. In addition, significant differences were observed between SA and P, SA and F, and SF and F. EPX appeared to be very resistant to degradation under flooded conditions. Patil et al., (1988) showed that 1-benzyltriazoles substitutes can be microbially degraded. As noted by Bromilow et al., (1999a), EPX molecule does not contain labile functional group easily broken by chemical processes. The EFSA Scientific report on EPX fate in soils (EFSA, 2008) also indicated that under anaerobic conditions (20°C), only 1.6 % of applied radioactivity as fluorophenyl-labelled EPX was mineralized after 120 days. EPX mineralization was also found to be low (< 5 % after 100 days) in a water/sediment study under flooded conditions using both 14C-U- chloro- and fluoro-phenyl labelled EPX (European Commission, 2006). In an aerobic degradation study of oxirane-labelled EPX in two soils, mineralization accounted for 7.6 to 22.5 % of applied radioactivity after 168 days. Our results were consistent with the available literature data showing that EPX has a very low ability to be mineralized in anoxic conditions. Parallel experiments have shown that EPX sorption was larger on SB and SF than on SA, although the differences were not significant (Chapter II:4). One of the main differences between SB and SA sediments was clay content, which was larger for SB (Table II-1). The higher sorption capacity in SB may be responsible for a lower EPX availability for mineralization. In addition, the SF soil, which also had similar clay content as the SB sediments, also showed a lower mineralization performance than the SA sediments. Mineralization was higher in the presence of vegetal substrates P and F than in SB sediments, but lower than in SA and SF. Consequently, the presence of such organic material in buffer zones may play a role in pesticide mineralization. After 177 days of incubation, it seemed that mineralization was still regularly increasing without reaching a plateau.

First-order models did not fit well mineralization kinetics. This was due to the fact that mineralization velocity did not significantly decreased nor reached any real plateau value during the 177-days incubation period. Only SB did not show any significant latent period

(Table II-6). This led to high (125 – 217 d) half-lives for EPX mineralization except for SB (74 d).

| Substrates | Mineralization | | | | | Disparition | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $C_{CO2max}$ | $k_{CO2max}$ | $\tau_{CO2}$ | $t_{1/2CO2}$ | $\sum e_i^2$ | $C_o$ | $k_{disp\ max}$ | $\tau_{disp}$ | $t_{1/2disp}$ | $\sum e_i^2$ |
| | % | $d^{-1}$ | d | d | - | $\mu g\ L^{-1}$ | $d^{-1}$ | d | d | - |
| SB[(a)] | 1.1 | 0.010 | 1 | 75 | 0.06 | 25.4 | 0.011 | | 63 | 16.4 |
| SA[(a)] | 8.2 | 0.007 | 250 | 438 | 0.14 | 25.2 | 0.018 | | 38 | 0.7 |
| SF[(a)] | 3.5 | 0.014 | 250 | 375 | 0.22 | 27.8 | 0.010 | 200 | 339 | 113.1 |
| P[(b)] | 3.8 | 0.006 | 250 | 467 | 0.04 | 24.2 | 0.026 | 250 | 340 | 16.8 |
| F[(b)] | 4.5 | 0.006 | 250 | 456 | 0.08 | 26.3 | 0.015 | 250 | 371 | 54.3 |

**Table II-6: Estimated mineralization and disparition kinetic parameters. [(a)] $\sum e_i^2$ are residues sum of squares. Mineralization parameters come from manually fitted modified lag-phase model. [(b)]SB and SA disparition parameters were obtained from first-order model whereas [(c)] SF, P and F disparition parameters were obtained from modified lag-phase models after lag-phases ($\tau_{disp}$) manually adjustment and $k_{CO2max}$ and $C_{CO2max}$ model fit.**

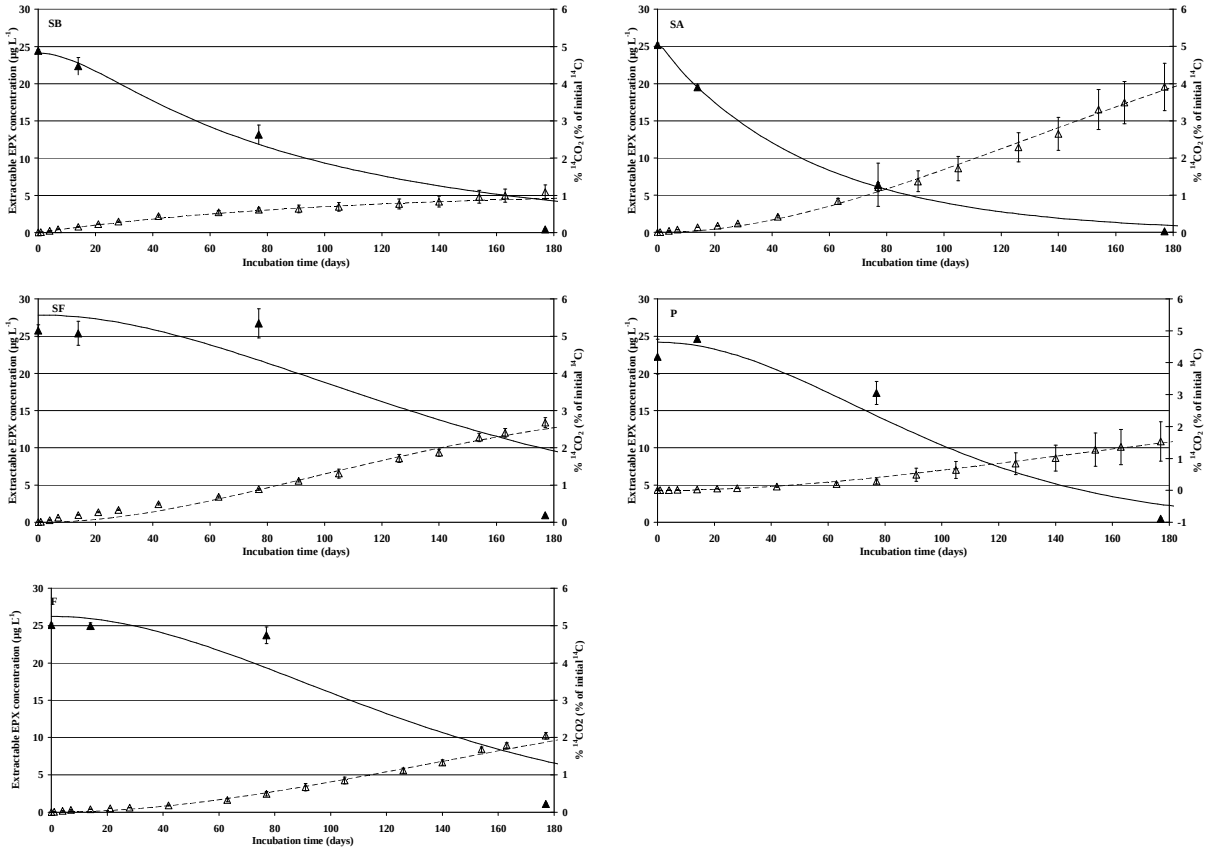


**Fig. II-6 : Disparition (left Y-axis) and mineralization (right Y-axis) of $^{14}$C-EPX. Full (▲) and empty (△) triangles represent measured EPX concentrations and $^{14}CO_2$ production, respectively. Full (disparition) and dashed lines (mineralization) are best fitted models.**

### 5.4.3 Extractable fractions

Mass balances on $^{14}$C-EPX showed that between 95.8 ± 1.5 and 126.3 ± 4.4 % of initially applied $^{14}$C-EPX was recovered at each time step. No significant loss of radioactivity was therefore noted.

Extractable fraction was a combination of water and methanol extractable radioactivity from substrates and water column. Water extractable fractions of radioactivity decreased from

the start to the end of the experiment for SB (Fig. II-7). It also decreased over the period corresponding to the first three measurement points (0, 14 and 77 days) but slightly increased again between days 77 and 177 for the other four substrates. Water could not extract radioactivity to a large extent, particularly for the wetland sediments and forest soil whose water extractable values were lower than 4 % of applied radioactivity. For forest litter and wetland plants, water extractable fractions varied between 4.2 ± 0.7 and 7.3 ± 0.6 % (F) and 13.3 ± 0.4 and 18.8 ± 1.8 % (P) along the incubation period. The most easily water extractable residues were observed on the substrates from which EPX was the most easily desorbed (P, F and SA), as discussed in the previous part of this chapter. The part of EPX that could be easily transferred back to water for possible degradation was low anyway. Contrary to water, methanol extracted a large part of the initial radioactivity with values ranging from 76.2 ± 0.2 to 87.7 ± 1.1 % on wetland sediments and forest soil at the end of the incubation period. On the two vegetal substrates, methanol extracts accounted for 76.2 ± 6.5 % (P) and 83.8 ± 2.0 % (F) of radioactivity. This indicated that EPX residues (parent or metabolite compounds) could potentially be mobilized.

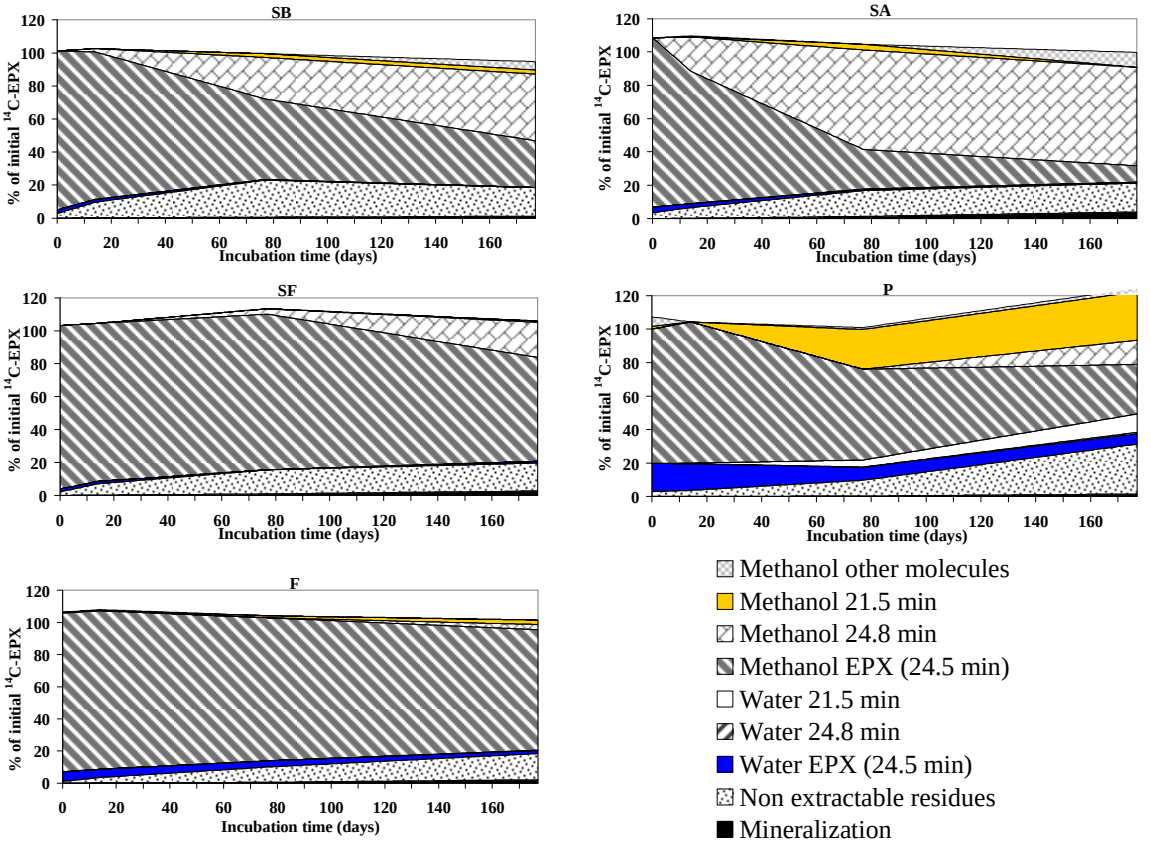


**Fig. II-7: Evolution of radioactivity distribution and composition for the five substrates. EPX parent compound was identified at 24.5 min whereas two other main peaks appeared at analytical times 21.5 and 24.8 min.**

Extractable radioactivity ($CaCl_2$ and methanol extracts) composition differed among substrates along the first 14 days of incubation (Fig. II-7). Water extracts only showed EPX and a compound at 24.8 min whereas methanol also extracted other molecules and one at 21.5 min. Over this period, more than 97 % of the initial radioactivity was made of EPX parent compound for SF, P and F. Lower values were already found for SB (91.0 ± 2.5 %) and SA (81.8 ± 2.2 %) at that time. These differences among substrates were maintained until the end of the incubation period. EPX fraction continued to decrease down to 28.3 ± 2.8 % (SB), 9.88 ± 1.14 % (SA) and 35.7 ± 3.6 % (P) at day 177. Conversely, SF and F extractable radioactivities at day 77 were still mainly made of EPX which finally accounted for 64.1 ±

4.7 % and 76.8 ± 5.3 %, respectively, at the end of the incubation period. At day 177, the complement in radioactivity for SA, SB and SF was mainly due to an unknown compound with a 24.8-min retention time, which was very close to EPX retention time (24.5 min), as shown in Fig. II-8 as an example. We therefore assumed that the new molecule had a form very similar to that of the parent compound. It is however difficult to affirm that it was an evidence of an EPX metabolite.

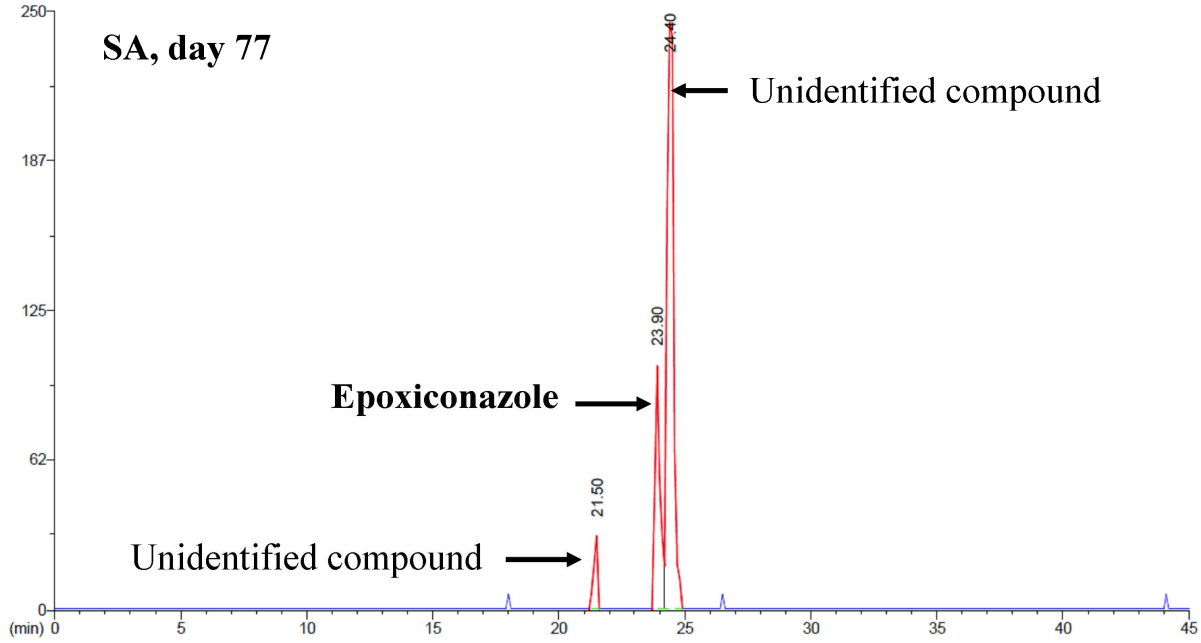


**Fig. II-8 : Example of a HPLC chromatogram for one of SA repetition at day 77 showing that radioactivity partitioned among EPX parent compound (herein at 23.90 min, in the text noted at "24.5") and two unidentified molecules at times 24.40 (noted "24.8" in the text) and 21.50 min.**

The EPX parent compound was a racemic mix of the two *cis*-enantiomers. Buerge et al. (2006) showed that epoxiconazole degradation was enantioselective in alkaline or slightly acidic soils, as were the sediments and forest soil of the present study. These authors noted a preferential degradation of one of the *cis*-enantiomers at alkaline pH, but did not notice any significant generation of pair *trans*-enantiomers. They observed EPX oxirane-ring cleavage and phenyl ring hydroxylation. However, the HPLC method we used could not make distinction between pairs of enantiomers. In the European commission draft assessment report on EPX (European Commission, 2006), one of cited studies was conducted under anaerobic conditions. After 120 days of incubation, traces of an alcohol and an alkene were detected, presumably generated after the cleavage of the EPX oxirane ring, successively leading to BF 480-alcohol and BF 480-entriazol alkene. However, they only accounted for a very small part of the initial radioactivity reaching 1.2 and 8.6 % of applied radioactivity, respectively, after 120 days of incubation. Water-sediments studies conducted for the EPX draft assessment report also showed the production of BF 480-entriazol EPX metabolite up to approximately 5 % and 30 % for an organic clayey loam and a non-organic sandy sediment, respectively (European Commission, 2006). Accordingly, the second chromatogram peak close to that of EPX observed in the present study may be that of the EPX-derived alkene. Another peak was noted at a 21.5-min retention time which only accounted for a small portion of the initial radioactivity (< 4%) for all substrates except P for which it reached 40.0 ± 8.3 % at day 177 for both water and methanol extracts (Fig. II-7). Patil et al. (1988) showed that triazoles substitutes, as the one attached to the $^{14}$C-oxirane ring of EPX, were microbially degraded. In addition, the EPX draft assessment report (European Commission, 2006) noted that 1,2,4-triazole degradates can be formed under aerobic conditions. The removal of the triazole group

from EPX represents a significant modification in the physico-chemical properties and a decrease in its molecular weight. Consequently, we assume that the peak observed at 21.5 min could correspond to the degradation product formed by the loss of the triazole ring. Triazole substitute groups are very soluble in water (730 g $L^{-1}$ for 1,2,4-triazole, 20°C) (FOOTPRINT, 2010). However, a study presented in the European draft assessment report showed that 1,2,4-triazole could also strongly bind to soil. The loss of this substitute group by microbial attack would confer the remaining molecule an even more hydrophobic character than EPX. However, water extractable fraction slightly increased between the last two time steps for plants and litter. Consequently, we could assume that at the end of the experiment, EPX unidentified metabolites appeared for SB, SA and mainly P. Plants exhibited a significantly larger water extractable fraction than the other substrates. However, this water extractable fraction decreased from 16.8 ± 1.8 to 13.3 ± 0.4 % between extraction days 14 and 77, respectively. It seemed that some microbial populations were able to degrade either totally or partially EPX in SB, SF, P and particularly in SA incubations. Conversely, with an intermediate mineralization rate, few metabolites were detected in the extractable fractions in F incubations. Wetland plants are made of fresher organic materials than forest litter. The former substrate may be more readily available to support micro-organisms growth thus presenting a larger ability to help EPX degradation than forest litter. However, overall, EPX degradation was slow but did occur under flooded conditions.

### 5.4.4 Non-extractable residues

In wetland sediments and forest soil, non-extractable residues (NER) rapidly increased during the first 77 days when they fairly reached a steady state until the end of the incubation period. SB, SA and SF NER were approximately 17 % of initial $^{14}$C-EPX applied at the end of the experiment (Fig. II-7). NER formation started quickly after the start of the experiment, particularly for SB which was also associated to a high microbial activity as soon as the experiment started. Plants and leaves also led to the formation of NER but at a slower rate than those observed in wetland sediments and forest soil. However, among the five substrates, plants showed the highest NER fraction (29.8 ± 6.6 %) at day 177, whereas that on forest leaves (16.3 ± 0.3 %) was close to those of SB, SA and SF. Soils exhibiting a high biodegradation potential are often associated to large fractions of NER (Alletto et al., 2006) and this is often enhanced when fresh organic material is present (Benoit and Barriuso, 1997; Benoit et al., 1999; Alletto et al., 2006). The EPX draft assessment report indicates the formation of 24.2 % of initial radioactivity (fluorophenyl labelled) as bound residues under anaerobic conditions. Approximately 20 % NER formation was observed in water/sediments studies using chlorophenyl and fluorophenyl labelled epoxiconazole (European Commission, 2006). These studies reported that the bound residues could be due to humic substances to which they were tightly bound. Plants adsorption potential was the lowest and led to the highest desorption rates (Chapter II:4). Meanwhile, our results indicated that fresh plant residues presented a greater capacity than litter or sediments or soil for enhancing NER formation. This suggests that an extended contact between EPX and plants may lead to the formation of NER inducing a reduced risk of pesticide transfer through wetland and forest can be expected with time of contact. When designing wetlands for pesticide pollution control, addition of plants should be encouraged to support water pollution reduction.

### 5.4.5 Disparition kinetics

Modified lag-phase models exhibited fairly good fits except for SF (Fig. II-6) for which kinetic parameters were less reliable. SB and SA had the lowest half-lives whereas those of the other three substrates ranged from 339 and 371 days (Table II-6). A half-life of 154 days was estimated from soil incubated under anaerobic conditions (European Commission, 2006). Bromilow et al. (1999a) compared a clay loam to a sandy loam soil by

estimating half-lives under different temperatures (5, 10 and 15 °C) and soil moisture contents (60, 80 and 100 % field capacity). They observed that EPX half-lives were very long ranging from 737 to 1540 days (> 2 years). However, those estimated on the clay loam soil were generally higher than those estimated on the sandy loam soil. These results demonstrate that wetland sediments would help degrading EPX more quickly than the forest soil or the vegetal substrates.

### *5.5 Conclusions*

Overall, under flooded conditions typically characterizing artificial wetland or forest buffer, very low epoxiconazole mineralization rates were measured. Non-extractable residues were formed in all incubations systems and were particularly important in the presence of wetland plants. Consequently, it can be thought that in artificial wetlands or forest buffer, the part of EPX that may come to sorbent proximity would adsorb on it. EPX adsorption potential may help it resist mineralization by reducing its availability to microorganisms. Vegetation exhibited a key role by enhancing epoxiconazole non extractable residue formation thus decreasing its mobility. It suggests that plant introduction should be advised while designing wetlands. Overall, artificial wetlands and forest buffers may reduce pesticide transfer risk from agricultural plots to receiving waters. However, long residence times will be needed to degrade hydrophobic molecules. Epoxiconazole was degraded into metabolites that were assumed not to be very different from the EPX parent compound. Their identification and toxicity characterization should be examined in the future. Buffer zones may provide complementary help to other actions targeting pollution reduction. However, they should not be considered the ideal and unique solution to eliminate such mineralization-resisting pesticides. As water level fluctuates, aerobic processes may take place. In the presence of higher oxygen concentrations, larger degradation rates are often measured which should also be part of further investigations to complement the present study. Favouring water level fluctuations (filling – emptying strategies) for oxygen transfer in wetlands and forest buffers may provide further degradation of pesticides.

### *5.6 Acknowledgements*

This research benefited from the FIRE (Fédération Ile-de-France de recherche sur l'environnement) and ArtWET LIFE (06/ENV/F/000133) financial supports. The authors also want to thank V. Dumeny for helpful laboratory work contribution.

## 6 General conclusions

The results obtained from these laboratory experiments (small space and small to intermediate time scales), conducted under controlled conditions, are supposed to have expressed maximal potential of wetland and forest buffer substrates to adsorb or degrade isoproturon, metazachlor and epoxiconazole. They demonstrated ability of these substrate to mitigate pesticides through partially reversible sorption processes. The incubation study showed that mineralization of epoxiconazole may be low and slow under flooded conditions. However, metabolites were observed suggesting that degradation process did take place.

These results must be complemented by additional experiments carried out on-site to get closer to the behaviour of pesticides under more realistic conditions, including water in movement. Such experiments, presented in Chapters III and IV, respectively include dynamic study of pesticide transfer, using on-site tracer experiments (intermediate time and space scales), and flow and pesticide concentrations and loads data acquisition from on-site continuous monitoring (large time and space scales).

# CHAPTER III: TRACER EXPERIMENTS FOR CHARACTERIZING SYSTEMS HYDRAULIC FUNCTIONING AND ON-SITE POTENTIAL FOR PESTICIDE POLLUTION MITIGATION

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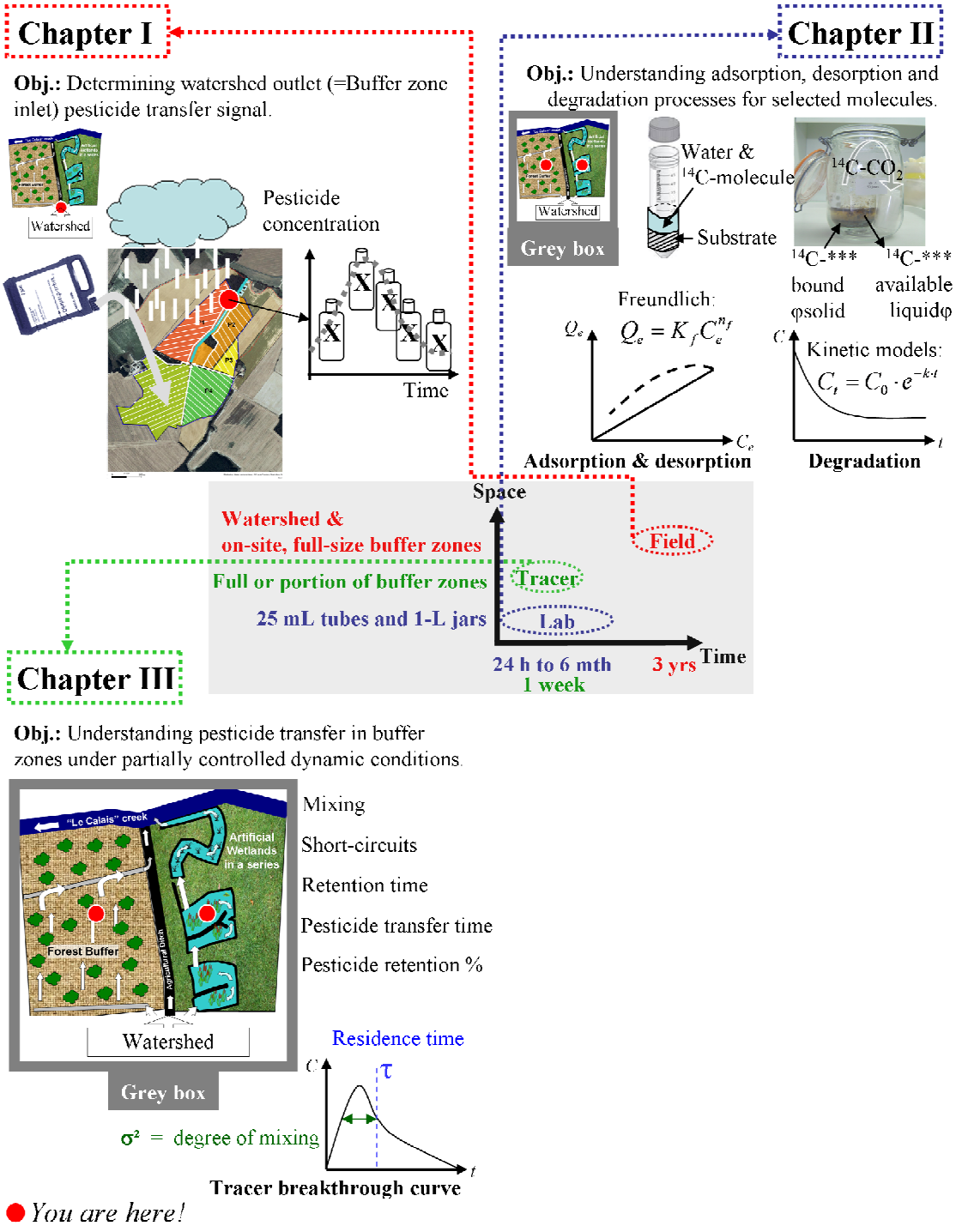


## 1 Introduction and objectives

Like any other kind of treatment system, buffer zones efficiency in improving water quality is highly dependent on their hydrological characteristics (Mitsch and Gosselink, 2000; Stearman et al., 2003). Water retention time within artificial wetlands and forest buffers is a key parameter. Indeed, the longer water remains in the systems, the more likely pesticides can undergo degradation or retention processes. Flow path short-circuits should be avoided as they reduce target pollutants interactions with sorption or degradation sites thus resulting in poor treatment (Mitsch and Gosselink, 2000). Other characteristics such as peak and volume reduction, peak delay, and performance variation with flood size may also impact system global performance (Strecker et al., 2000). In artificial wetlands, macrophytes can provide pollutant sorption sites and microbial growth support (Stottmeister et al., 2003; Kadlec and Wallace, 2008). Vegetation distribution is of importance as it affects water and pollutants velocity (Rose et al., 2008). Jenkins and Greenway (2005) showed that fringing vegetation (located on wetland edges) could accelerate water flows in central zones thus decreasing retention time and system treatment efficiency.

There is little literature concerning pesticide fate from on-site wetland or forest buffer studies under partially controlled conditions. Pesticide inlet versus outlet concentration or load reductions were studied in outside wetland mesocosm systems (Moore et al., 2000; Moore et al., 2001b; Moore et al., 2002; Braskerud and Haarstad, 2003; Haarstad and Braskerud, 2005), and forested areas (Lowrance et al., 1997b; Vellidis et al., 2002; Gay et al., 2006). However, detailed concentration monitoring procedures have rarely been implemented (Matamoros et al., 2007; Kidmose et al., 2010).

Despite presenting high potential for pesticide dissipation in forest buffers and artificial wetlands, results from the previous laboratory experiments (Chapter II) are not immediately transferable to field scale because water flow dynamics, substrate to water depths ratio, oxygen concentrations, temperature… are much more variable on-site than under laboratory controlled conditions. Before going over results from on-site buffer zones efficiency assessment (Chapter IV), tracer experiment techniques were selected to approach pesticides and water flow dynamics under partially controlled experiments at intermediate time (a few days to a few weeks) and space (portion of forest buffer and whole artificial wetland) scales.

Tracer experiments are useful techniques to characterize wetland systems internal hydraulics, including residence time and degree of mixing (Kadlec and Wallace, 2008). Among conservative tracers, salts (NaCl, LiCl, KBr) and fluorescent dyes (uranine, sulforhodamine) have been commonly used (Mortensen et al., 2004; Dierberg and DeBusk, 2005; Maloszewski et al., 2006).

The two tracer experiments presented in this chapter were carried out in March 2008 and March 2009. The first tracer experiment, conducted through the LIFE ArtWET project in collaboration with the Institute for hydrology (Freiburg, Germany), has been described in two papers (Passeport et al., 2010b; Lange et al., In Press). It included fluorescent tracers' injection concomitantly with a mobile herbicide isoproturon, thus placing March 2008 study in a worst-case scenario for pesticide fate study in an artificial wetland and a forest buffer. The second tracer experiment (March 2009) was conducted in the forest buffer with bromide and six pesticide molecules to understand the fate of pesticides presenting different physicochemical properties. It was carried out through a master student project (Richard, 2009), in collaboration with the Cemagref Lyon through a convention with DGPAAT ("Direction Générale des Politiques Agricole, Agroalimentaire et des Territoires", French Ministry of Agriculture and Fishing). Buffer zone hydrological assessment was evaluated by calculating hydraulic metrics.

## 2 Site description

### *2.1 Artificial wetland and forest buffer*

Two buffer zones, an artificial wetland (AW) and a forest buffer (FB) system (Fig. III-1), were constructed in late 2007 for pesticide pollution mitigation on the lower portion of the watershed (Fig. I-1). The artificial wetland was constructed above a previously artificially drained plot whereas the forest buffer was not. Remaining pipe drainage located under the wetland ensured a diameter-limited leakage flow rate (not exceeding 0.5 L/s) to slowly empty out the system. This drainage outlet was blocked from 03 Dec. 2008 as described later (section 2.3).

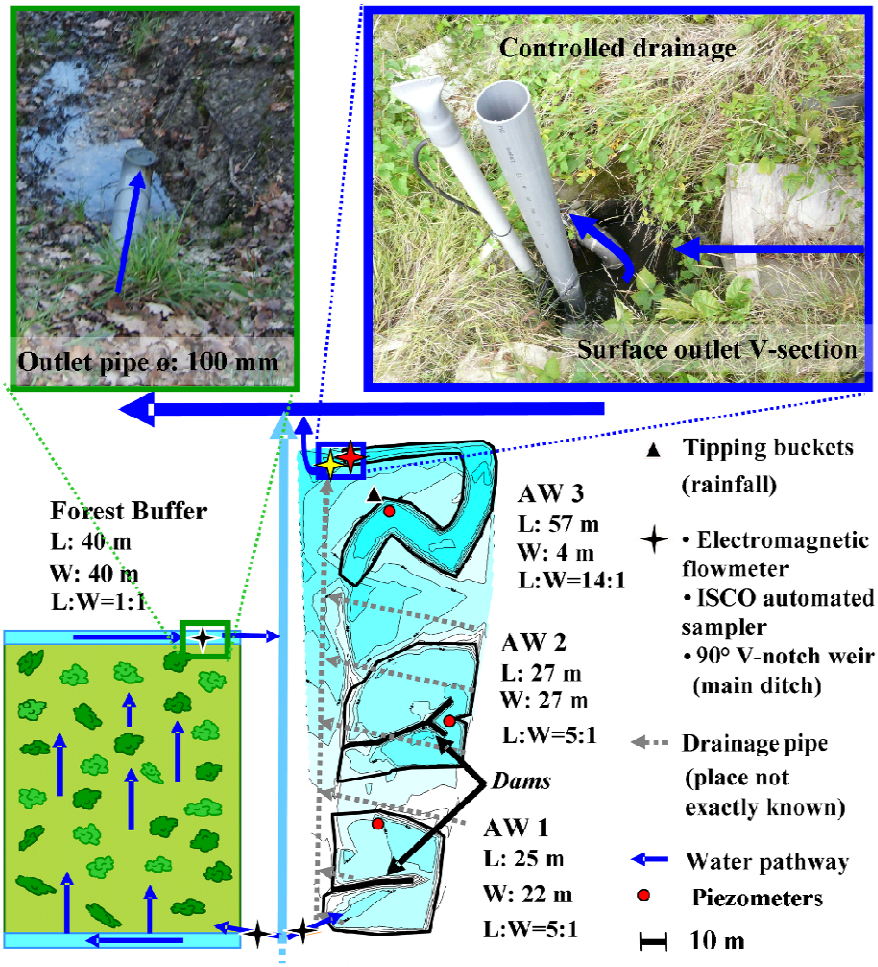



**Fig. III-1: Buffer zone map showing monitoring equipment and remaining drainage system (AW1, AW2 and AW3 stand for artificial wetland 1, 2 and 3, respectively, from the higher to the lower part of the watershed). Topographic line spacing is 20 cm. The yellow and red stars at the wetland outlets are drainage and surface outlets, respectively. Dotted grey arrows show drains and drain collector locations. The drainage outlet was definitely blocked on 3 December 2008 (see section 2.3).**

The artificial wetland consisted of three cells in a series referred to as AW1, AW2, and AW3 from the upper to the lower part of the area as shown in Fig. III-1. Both AW1 and AW2 comprised a dam to lengthen the water flow path and reduce short-circuits. This led to apparent length-to-width ratios of 5:1 for AW1 and AW2, whereas AW3 had a more tortuous path with a L:W ratio of 14:1 (Fig. III-1). Water runs off through grassed areas between AW1 and AW2, as well as AW2 and AW3. The wetland's total surface area was 1280 m² and the total storage volume capacity was approximately 330 $m^3$. Each cell maximum depth was 0.70,

0.77, and 0.14 m for AW1, AW2, and AW3, respectively. Water level was continuously recorded every 15 min from 27 Nov. 2007 by means of automated probes (MADOFIL, Iris Instruments, France). The underlying upper 20-cm soil layer had a silty clay texture (Table III-1) and presents typical redoximorphic features due to water level fluctuations generating varying redox potential (Fig. III-2).

**Fig. III-2: Wetland soil presenting redoximorphic features (red oxidized iron and blue-grey reduced iron presence, typical of redox potential variation)**

In the forest buffer, water runs off according to a sheet-flow presenting extended contact with forest soil and litter. The forest soil's clay content increased from 15 cm to 45 cm in depth, up to 37 % limiting forest soil infiltration capacity (Table III-1). The forest buffer slope was 1.38 % approximately.

| | Artificial wetlands | Forest buffer |
|---|---|---|
| | AW | FB |
| Global length-to-width ratio (L:W)† | 20:1 | 1:1 |
| Surface area ($m^2$) | 1280 | 1600 |
| % Watershed surface area | 0.30 | 0.40 |
| Storage volume capacity ($m^3$) | 330 | - |
| Clay (%)†† | 36.2 | 26 |
| Silt (%)†† | 53.1 | 49.6 |
| Sand (%)†† | 10.7 | 24.4 |
| Organic matter (%)†† | 2.7 | 8.16 |
| Soil texture type†† | Silty clay loam | Silt loam |

**Table III-1: Buffer zones' main characteristics. † Length-to-width ratios for each artificial wetland cell were 5:1 (for AW1 and AW2) and 14:1 (for AW3). †† 0 – 20 upper cm soil composition.**

Each buffer zone was located parallel to the main ditch and each surface area accounted for less than 0.5 % of the watershed area, which maximized the mitigation system's surface areas considering land availability. In addition, the buffer zones were located in between a creek called "Le Calais" and the small (46-ha) Bray headwater catchment. They received less diluted flows than those coming from larger watersheds. At such a reduced scale, pesticide loads and water flows are closely related to pesticide applications and rainfall events (Chapter I). These buffer zones are therefore likely to receive higher loads in lower volumes compared to those from larger-scale watersheds. It has been shown previously that wetland efficiency sometimes increases (Moore et al., 2001b) or remains high (Schulz and Peall, 2001; Moore et al., 2002) for high inlet pesticide concentrations. Because of limited land availability, downstream buffer zones could not accommodate all water volumes from the watershed. Consequently, a strategy implying two techniques was tested. First, buffer zone inlets were

limited by means of a PVC pipe measuring 200 mm (forest buffer before 18 Feb. 2009 and artificial wetland) or 100 mm (forest buffer, after 18 Feb. 2009) in diameter, corresponding to a maximum flow rate of approximately 35 L/s (200 mm) or 9 L/s (100 mm) (see chapter I), respectively. This made it possible to focus on the first rising part of the floods that may contain the maximum pesticide loads (Branger et al., 2008). Second, based on conclusions from Chapter I, the systems were managed by the farmer who was in charge of opening and closing the water entrance in both buffer systems according to pesticide applications. Indeed, even if it was demonstrated that not only flows following pesticide applications are of most concern, these are usually associated with high pesticide concentrations. During specific periods following pesticide applications, pipe drainage waters were split into two parts and diverted through each mitigation systems' inlets. The "open – close strategy" consisted in opening the systems for approximately one month after pesticide applications and then closing them to give time to caught water volumes and pollutants to undergo dissipation processes. For that purpose, two PVC pipes linked the agricultural main ditch collecting drainage water from the 46-ha watershed, to the forest buffer (on the left-hand side of the main ditch) and the artificial wetland (on the right-hand side of the main ditch) (Fig. III-3). In the main ditch, these PVC pipes were associated with elbows that could be turned up or down. Turned up, it prevented water from entering the systems ("close" position), whereas, down-turned elbows enabled flow towards the buffer zones ("open" position). In Fig. III-3, the forest buffer is closed and the artificial wetland is open.

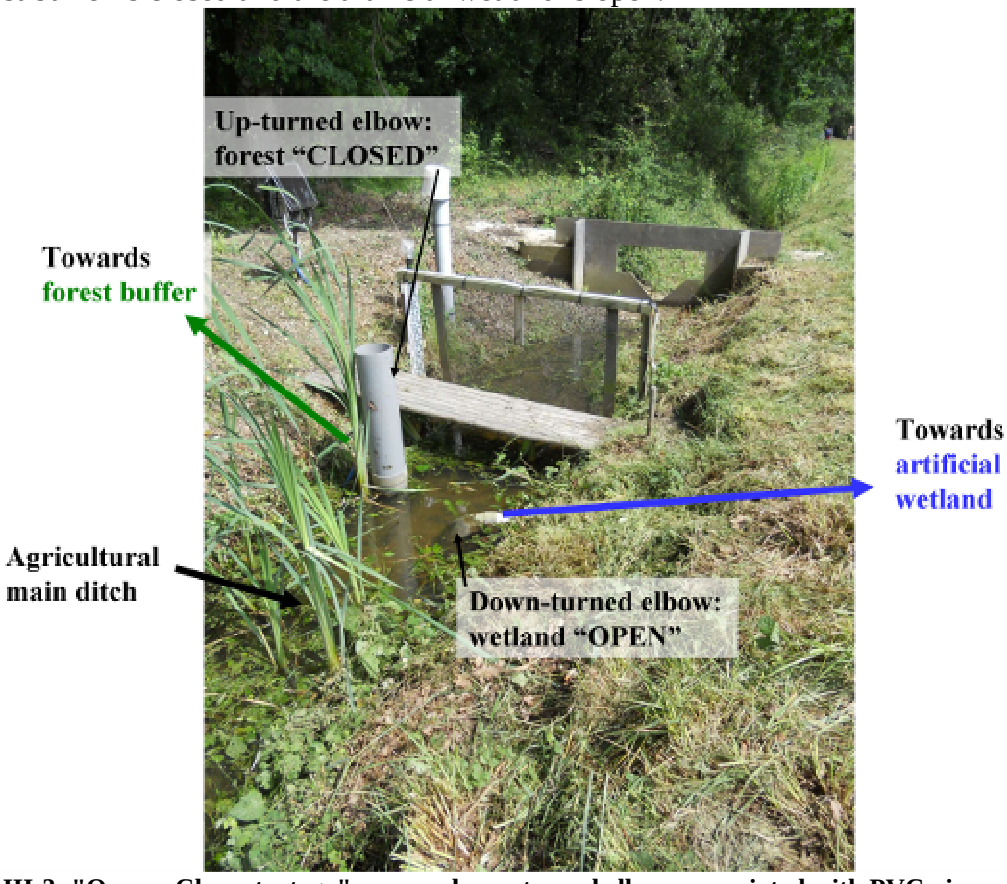



**Fig. III-3: "Open – Close strategy": up- or down- turned elbows associated with PVC pipes enable to close or open the buffer zones, respectively.**

Both systems' outlets fed water into the natural creek. The artificial wetland comprised two distinct outlets corresponding to surface (at the outlet of AW3) and pipe drainage collector outlets (Fig. III-1). The surface outlet was located at the outlet of a collecting ditch made impervious thanks to an EPDM coating (from Fireston, France). The pipe drainage remaining under the cells of the artificial wetland was also assessed for its function in emptying the wetland. It was definitively controlled (blocked) from 03 Dec. 2008 as

explained in section 2.3. From previous conclusions dealing with treatment wetland design (Kadlec and Wallace, 2008), AW1 was expected to provide most of the sedimentation, whereas AW2 was intended to specifically enhance pesticide sorption. Finally, photodegradation and biochemical degradation could occur in each artificial wetland cell where vegetation shade was limited. AW3 mainly comprised grass (*Festuca arundinacea*) and behaved like the more usual buffer zones with low temporary water levels.

*2.2 Vegetation*

The wetland had been planted in 2006 with 75 *Glyceria maxima*, 75 *Carex pseudo-cyperus*, 50 *Iris pseudo-carpus*, 50 *Filipendula ulmaria*, 10 *Juncus conglomeratus* and sown in *Festuca arundinacea*. It has been dug again in December 2007 to increase wetland surface area and water storage capacity. The first 20 cm of the wetland soil was reserved during the works and spread out back to the wetland once construction was over to provide the wetland clayey soil a more organic and suitable substrate for plants to grow. No additional planting was carried out. Vegetation colonization therefore started again on its own. The artificial wetland vegetation evolution from wetland reconstruction (December 2007) to the end of the monitoring period is pictured in Fig. III-4.

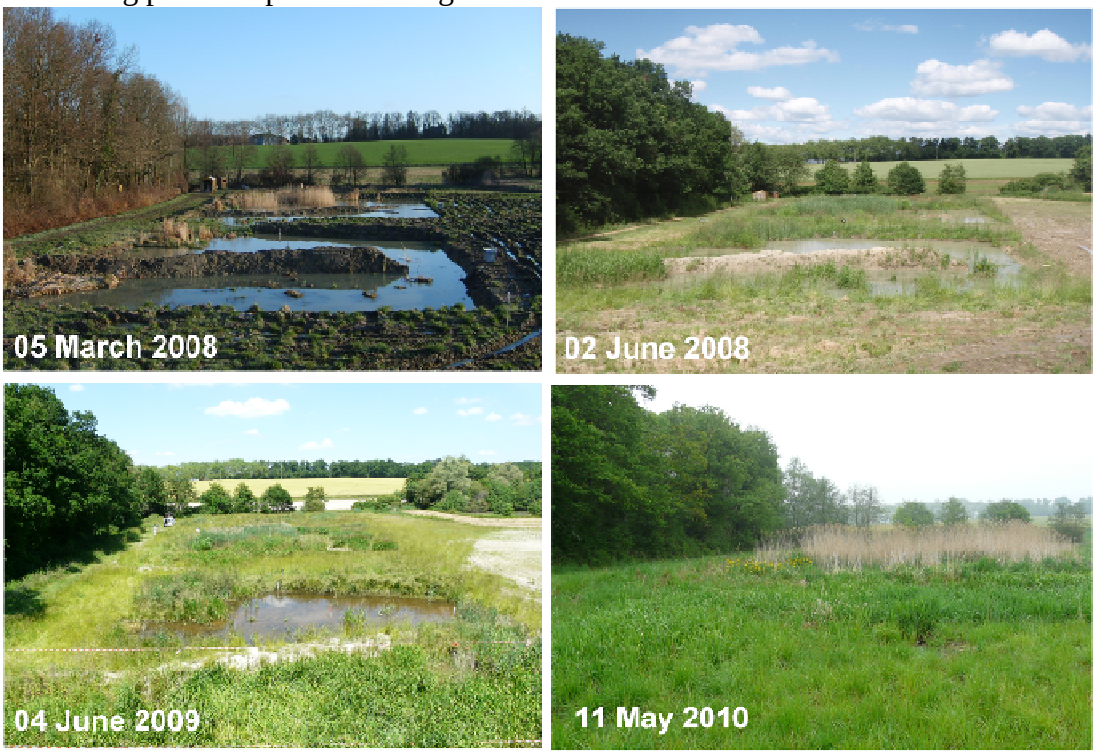



**Fig. III-4: Artificial wetland vegetation evolution along the monitoring period**

Vegetation species identification and percent coverage calculations were determined in spring 2009 by two first-year master students (Blin, 2009; Meigné, 2009) and the help of Franck Paineau (GDA Loches-Montresor, France) and Francesca Di Pietro (Univsertity of Tours, France). Sampling locations were randomly determined based on a grid overlapping the artificial wetlands. The grid was made of horizontal and vertical transects 7-m spaced out in both directions. Transects intersections corresponded to 1-m² quadrat center positions. Some quadrats were slightly moved taking into account on-site topography (red circles in Fig. III-5). Two persons were systematically working together on each quadrat from 2 to 4 June, 2009 and visually estimated flora species and percent coverage. Only flooded quadrats were considered. Free surface water, emerged soil and unplanted soil percentages were also recorded. Previous visits had been organized (7, 14 and 28 May 2009) to determine the best sampling strategy and get familiar with plant species, particularly thanks to the help of Pr. J. Haury from AgroCampus (Rennes, France).

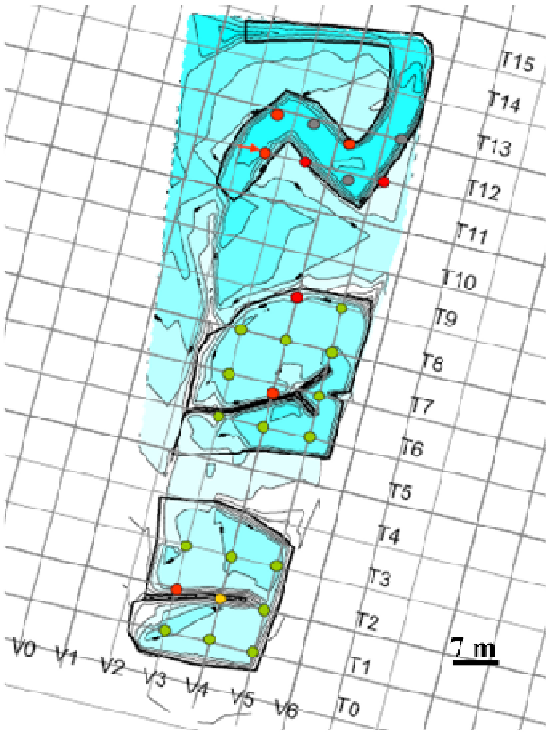



**Fig. III-5: Quadrats localization (circles) for wetland flora inventory. Green circles are quadrats located in ponded zones water; grey circles are those located in water-free zones; yellow circle is that originally positioned on the embankment whereas red circles are those moved on the embankment from their original position because of accessibility difficulties or slight errors while measuring distances on site (from Meigné (2009)).**

Flora inventory indicated that there is no endangered species and a dominance of tall aquatic plants like *Glyceria maxima* (53.2 %), *Festuca arundinacea* (11.8 %), *Phragmites australis* (10.0 %) and *Phalaris aundinacea* (8.8 %) as shown in Fig. III-6 and Fig. III-7. Such a vegetation distribution is typical of treatment wetlands in agricultural landscapes where nitrogen is not limited (Guntenspergen et al., 1989). Nitrate rich waters, as are drainage waters from agricultural watersheds, may help the development and predominance of plants like *Phalaris arundinacea* (Green and Galatowitsch, 2002).

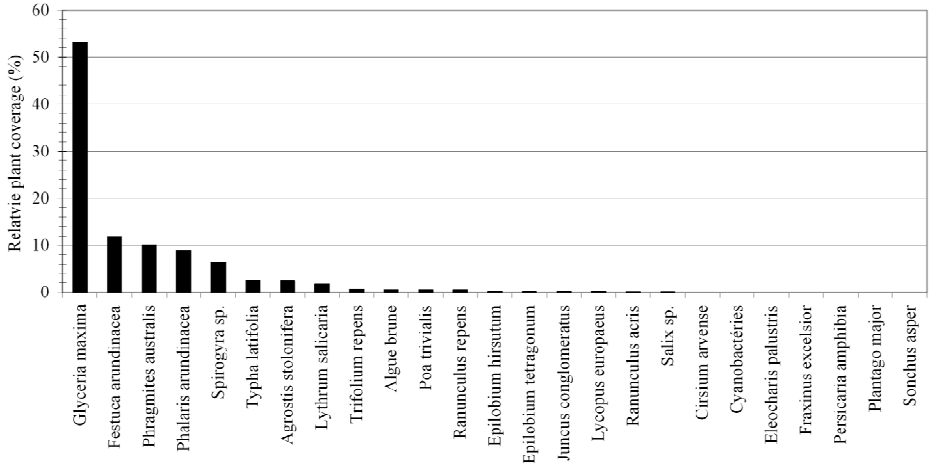
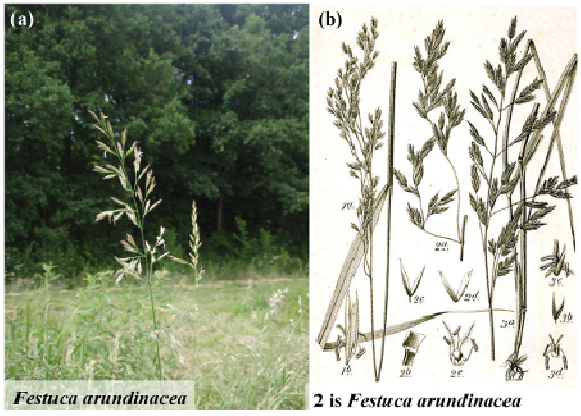



**Fig. III-6: Plant species and relative coverage (%) at the Bray artificial wetland on 4 June 2009.**


(1)

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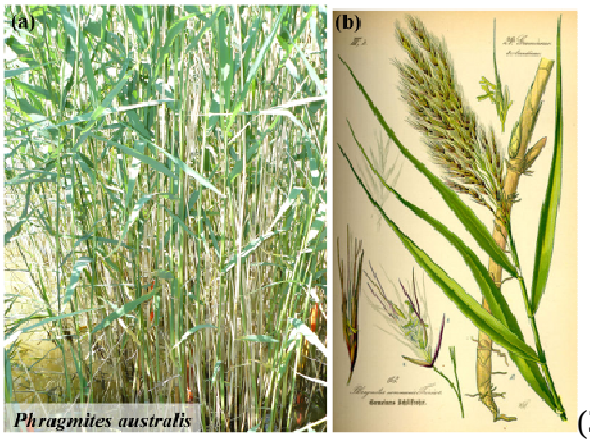


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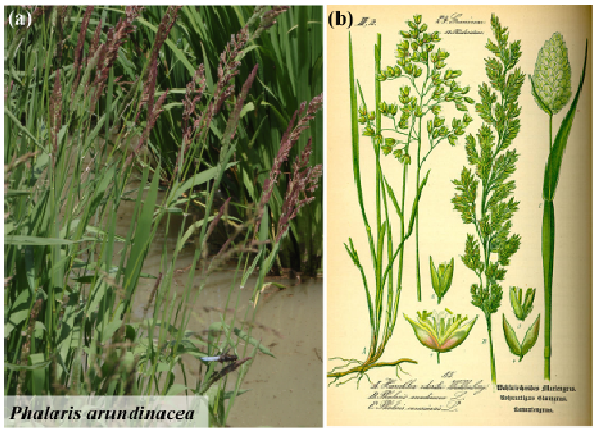



(4)

**Fig. III-7: (a) Photo of plants taken at Bray early June 2009 and (b) drawings from www.biolib.de (except for Phalaris arundinacea found on http://deoxy.org/, on 20 Aug. 2010) corresponding to (1) *Glyceria maxima*, (2) *Festuca arundinacea*, (3) *Phragmites australis* and (4) *Phalaris arundinacea*.**

On the other side of the ditch, a 1600-m² forest buffer mainly made of common oak trees (*Quercus robur*) (Fig. III-8) stands on a silt loam soil (Table III-1).


**Fig. III-8: Forest buffer main vegetation (*Quercus robur*). Photos are from the Bray forest buffer and drawing is from www.biolib.de.**

### *2.3 Underlying drainage*

Because they were acting as short-circuits, remaining subsurface drains were attempted to be blocked. During wetland reconstruction works in December 2007, two drains located under AW1 were accidentally cut. They were manually clogged with clayey soil. Digging works to increase wetland volume therefore implied that remaining drains were closer to wetland soil surface than before these complementary works. On 26 November 2008, a hole was dug at the wetland outlet (yellow star in Fig. III-1), right by the collecting manhole receiving artificial wetland surface and drainage outflows. The 80-mm diameter drain collector was intercepted, cut and clogged with cement on 27 Nov. 2008 (Fig. III-9). In addition, an up-turned PVC pipe was adapted to cut drain collector outlet in the manhole thus helping blocking subsurface drain outflows. However, observations of puddles between AW2 and AW3 and along AW2 indicated partial failure of this unique controlled structure. Consequently, a second intervention was carried out on 3 Dec. 2008. Drain collector was cut on 2-m long sections at its intersections with four connected drains located up- to down-stream AW2. Collector was clogged at each cut section with cement (Fig. III-10).

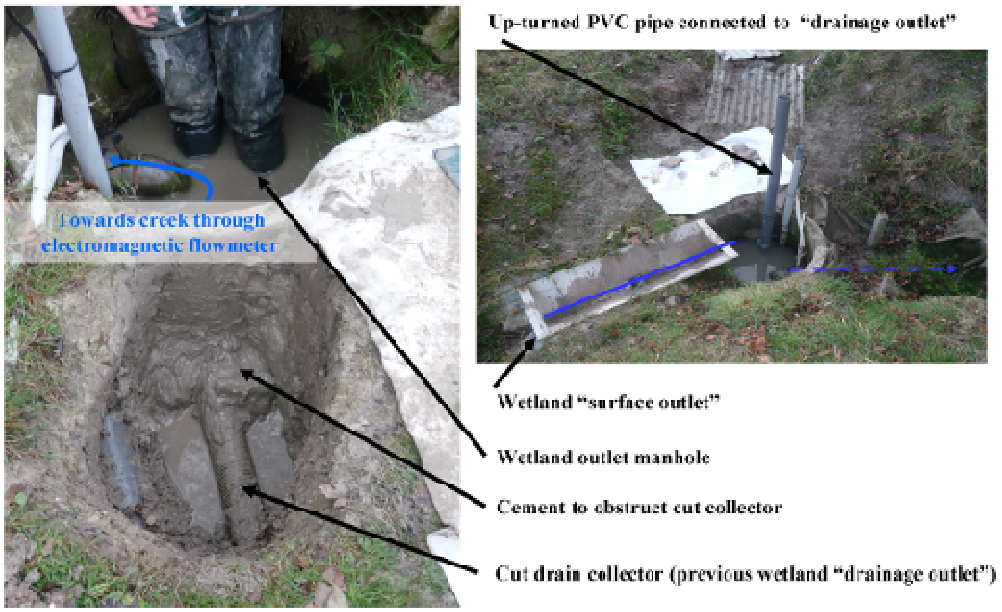



**Fig. III-9: Artificial wetland outlet drainage collector interception, cutting and block with cement on 27 Nov. 2008.**

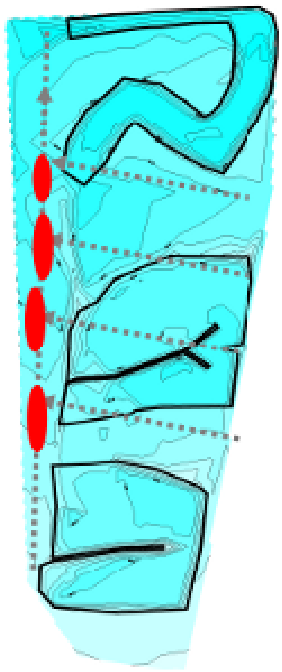


**Fig. III-10: Controlled drainage conducted on 3 Dec. 2008. Dotted grey arrows show drains and collector location. Red points indicate where on the collector drains were disconnected by cutting and clogging with cement the collector at four different locations.**

## 3 Materials and methods

### *3.1 March 2008: Tracer experiments in artificial wetland and forest buffer*

#### 3.1.1 Multi-tracer experiment conditions

A multi-tracer experiment was conducted from 5 to 10 March 2008 to determine the main water flow paths and water residence times of the mitigation systems and to simulate the fate of molecules presenting contrasting properties. This period was approximately 3 months after the artificial wetlands were reconstructed to increase their sizes and volumes. The tracer experiments were conducted in the whole artificial wetland (330 $m^3$, 1280 $m^2$) but in a reduced portion of the forest buffer (530 $m^2$) delimited with man-made levees.

A pulse injection took place on 7 March 2008 at 09:45 at the forest buffer inlet (time after the tracer injection into the forest buffer, $tati_{fb}$ = 0) and on 5 March 2008 at 17:24 at the inlet of the artificial wetland (time after tracer injection in the artificial wetland, $tati_{aw}$ = 0). The forest had been opened on 7 March, 09:19 ($tati_{fb}$ = −0.4 h), just before the injection, and for one full night from 6 March, 16:15 ($tati_{fb}$ = −17.5 h) to 7 March, 08:57 ($tati_{fb}$ = −0.8 h) during which water ran off through the experimental area. The forest buffer had been closed by the farmer since 10 January 2008, 16:45, but was open again one day before the tracer experiment took place. The artificial wetland had been closed at the time the forest was injected with tracer ($tati_{aw}$ = 40.3 h) to obtain a higher flow rate at the forest buffer inlet. The artificial wetland was opened again on 7 March at 16:55 ($tati_{aw}$ = 47.4 h). Injected molecules consisted of a water dye tracer, sulforhodamine B (2-(3-diethylamino-6-diethylazaniumylidene-xanthen-9-yl)-5-sulfo-benzenesulfonate, SB, $C_{27}H_{29}N_2NaO_7S_2$), one dye tracer molecule simulating photodegrading pesticides, uranine (Ur, $C_{20}H_{10}Na_2O_5$), and a herbicide usually applied on site, isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea, IPU, $C_{12}H_{18}N_2O$).

Uranine is anionic for pH values greater than 5.05 (Franke et al., 1997). This molecule is known to photodegrade easily, whereas sulforhodamine B is not light-degradable (Smart

and Laidlaw, 1977). Uranine and sulforhodamine B adsorption properties are closely related to the media. Uranine presents low sorption coefficients on negatively charged surfaces (Käss, 1994; Li et al., 1998; Kasnavia et al., 1999), whereas sulforhodamine B normally shows higher sorption but still lower than other rhodamines (Käss, 1998). Both fluorescence dye tracers have been previously used for wetland and pond hydraulic characterization (Torres et al., 1997). Both dyes are usually employed simultaneously to facilitate this task given their different absorption spectrum. Isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea, IPU) is a selective herbicide inhibiting photosynthesis. Its $K_{oc}$ ranges from 36 to 241 mL/g (INRA, 2010) whereas isoproturon half-life ($DT_{50}$) values range from 12 to 33 days (Calvet et al., 2005b). Isoproturon is used on winter cereals such as wheat and barley from October to January.

The initial 20-L tracer solution was prepared in a plastic bucket with on-site water and pre-weighted tracer masses. A pump was used to inject the tracer solutions at the buffer zone inlets at a 0.185-L/s flow rate. Subsequently, additional 60 L of rinsing water was poured into the systems to ensure total tracer mass injection and mixing. Contrasting hydraulic conditions were obtained throughout the experiment. Indeed, an almost steady-state low flow was present from the injection until 10 March, 14:00, averaging 1.4 L/s and 0.2 L/s at the artificial wetland and the forest buffer inlets, respectively. This corresponded to good natural flow conditions to carry out a tracer experiment. Because of a very low inlet flow rate at the time of the experiment, only a portion of the forest (approximately 530 m²) was used. On the other hand, the artificial wetlands were full of water prior to injection.

### 3.1.2 Materials and analysis methods

Buffer zones inlet and outlet flow rates were monitored with electromagnetic flow meters (MAG 8000 Siemens, HYDREKA, Saint Cyr au Mont d'Or, France) (see Fig. I-5 and Fig. III-11). In addition, a V-notch section was installed on 30 January 2008 to specifically monitor artificial wetland surface outflow rates. This was done by means of a PDCR1830 pressure transducer (Druck, Asnières, France) for water level measurement set up on 4 March 2008. This helped distinguishing between surface and drainage outlets before drainage was blocked (3 December 2008).

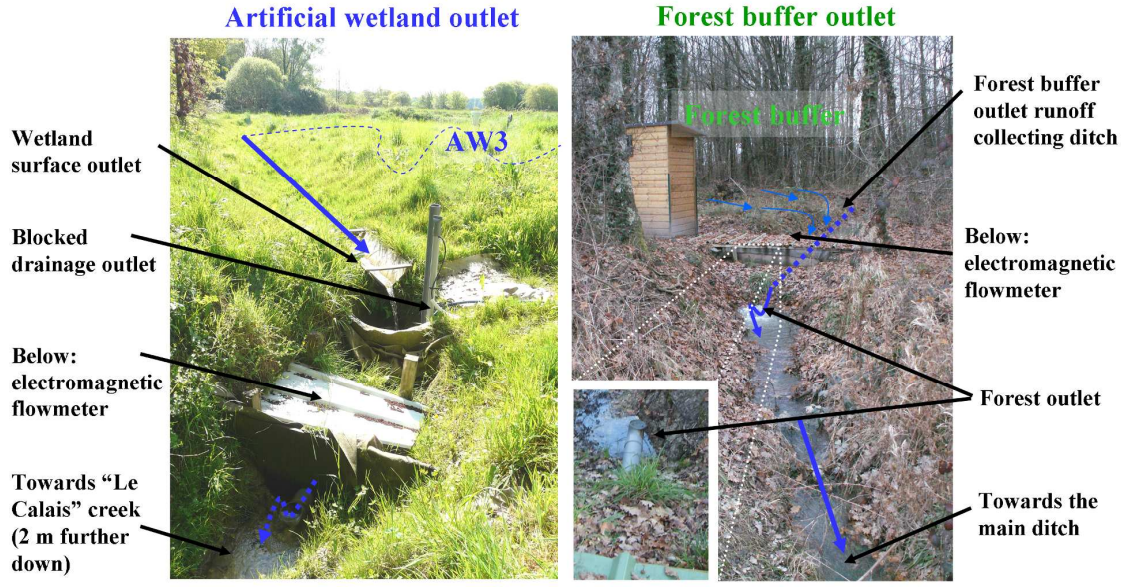
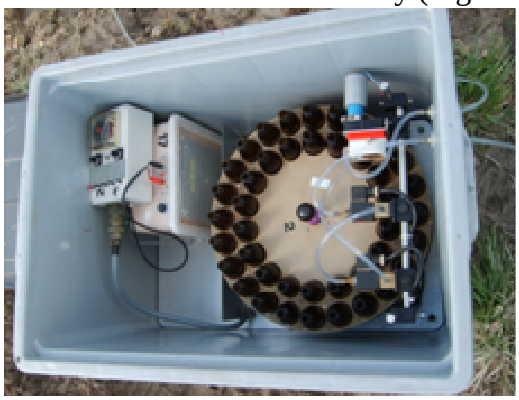



**Fig. III-11: Artificial wetland and forest buffer outlets. Blue arrows represent flow paths.**

Multi-flasks automated samplers (Fig. III-12) were installed at the outlets of each artificial wetland cell, the remaining buried drainage system under the wetlands, and the

forest buffer. Samples were collected every 1–4 h in brown 100-mL glass bottles used for uranine and sulforhodamine B analyzes. Two-milliliter subsamples were transferred to brown glass vials for isoproturon analyzes. Two FL30 filter continuous fluometers, located at AW3 surface and pipe drainage outlets, enabled recording uranine and sulforhodamine B concentration data continuously (Fig. III-12).



(a)

(b)

**Fig. III-12: (a) multi-flasks automated samplers and (b) FL30 filter continuous fluometer, from the Institute for Hydrology, Freiburg, Germany.**

After the experiment, time-dependent samples were analyzed by means of ELISA immunoassay tests (Envirologix[tm]) for isoproturon (Mouvet et al., 1997). Sulforhodamine B and uranine concentrations were determined using a Perkin Elmer LS 50 B luminescence spectrometer.

Sampling time steps (30 min to 4 h) were longer than those of flow rate records (15 min). In addition, it should be noted that an 8-h frost period starting on 6 March 2008, 01:00 ($tati_{aw}$ = 7.5 h) prevented from sampling in the artificial wetland. Linear regression interpolations were employed to estimate concentrations for nonsampled time-steps. At AW2 and pipe drainage outlets, concentration distribution tails did not reach zero. To face this issue for residence time calculations, concentration data were extrapolated by means of an exponential decay function determined from the values obtained after the last curve inflection. From tracer concentration observations and field knowledge, it was assumed that 80% of subsurface drainage flows could be attributed to AW1 and 20% to AW2. Therefore, AW1 and AW2 outflow rates were estimated by individual mass balances considering AW inlet flow rates, the rainfall contribution on each wetland surface area, and pipe drainage outlet.

### 3.1.3 Residence time calculations

Loads and tracer recovery rates were calculated for each artificial wetland cell and at forest outlet for each tracer by means of equations 17 and 18 below:

$$m_i = \int_0^{t_i} Q(t)C(t)dt + \int_0^{t_i} p * Q_d(t)C_d(t) \qquad \text{(Eq. 17)}$$

$$R = \frac{m_{out}}{m_{in}} \times 100 \qquad \text{(Eq. 18)}$$

where:

$m_i$: total cumulated mass at time $t_i$ after injection (mg)

Q(t): surface outlet flow rate (L/s)

C(t): concentration at the surface outlet (mg/L)

*p*: proportion of the artificial subsurface drainage attributed to the corresponding artificial wetland cell ($p$ = 0.8 for AW1, $p$ = 0.2 for AW2, $p$ = 0 for AW3 and the forest buffer)

$Q_d(t)$: artificial subsurface drainage flow rate (L/s)
$C_d(t)$: artificial subsurface drainage concentration (mg/L)
$m_{out}$: outlet mass (mg)
$m_{in}$: inlet mass (mg)
R: recovery rate (%)

As proposed by Lange et al. (In Press), specific tracer retention (*STR*) were calculated normalizing percent reduction (100 - *R*) to wetland volume. For the forest buffer, percent reductions were normalized to forest buffer surface area, and noted *ATR*. This was proposed to facilitate comparisons among tracer experiments conducted in systems of different sizes. Large values of *STR* and *ATR* express that small system volume or area contribute to high tracer load reduction. Each tracer molecule has its own travel time to reach the facility outlet. A distribution of residence times thus exists in wetland systems, which is a probability density function (E(t)) known as the residence time distribution (RTD) (Kadlec and Knight, 1996). For steady-flow conditions, the E(t) function can be written as:

$$E(t) = \frac{Q(t)C(t)}{\int_0^\infty Q(t)C(t)dt} \quad \text{(Eq. 19)}$$

The artificial wetland cell mean residence time ($\tau$) was determined from the first moment:

$$\tau = \int_0^\infty tE(t)dt \quad \text{(Eq. 20)}$$

The system scale of mixing is described well by the spreading of the tracer response curve around the mean of the distribution ($\tau$) given by the second central moment, also known as variance ($\sigma^2$):

$$\sigma^2 = \int_0^\infty (t-\tau)^2 E(t)dt \quad \text{(Eq. 21)}$$

A dimensionless variance (square of coefficient of variation, $\sigma_\theta^2$) is calculated according to the following equation:

$$\sigma_\theta{}^2 = \frac{\sigma^2}{\tau^2} \quad \text{(Eq. 22)}$$

In addition to the mean residence time ($\tau$) and the degree of mixing ($\sigma^2$ or $\sigma_\theta^2$), different metrics can be used to determine hydraulic efficiency. Each of them will be related to either short-circuits or mixing. They must all be considered to assess wetland hydraulic efficiency (Holland et al., 2004). A relationship was developed by Thackston et al., (1987), who defined the effective volume ratio (e) calculated by the ratio of the mean to the nominal residence times ($T_n$):

$$e = \frac{\tau}{T_n} \quad \text{(Eq. 23)}$$

Nominal residence times, $T_n$, were defined as the ratio of the total surveyed wetland volume to the average flow rate at the surface outlet of each wetland. Because of dead zones and preferential flow paths or short-circuits, the *e* parameter provides a first indication on how far the system deviates from an ideal flow. In case the selected tracer would not perfectly simulate water flow path by being sorbed and subsequently released, the *e* parameter could be higher than unity.

To better describe wetland hydrodynamics, Persson et al., (1999) proposed a second parameter referred to as hydraulic efficiency ($\lambda$), which describes the breakthrough curve shape introducing a number of tanks in series (N) to describe the spreading of the tracer:

$$\lambda = e\left(1-\frac{1}{N}\right) = \left(\frac{\tau}{T_n}\right)\left(1-\frac{\tau - t_p}{\tau}\right) = \frac{t_p}{T_n} \qquad \text{(Eq. 24)}$$

In the previous equation, N is the number of continuously stirred tank reactors (CSTRs) in a series and $t_p$ represents the time to peak outflow concentration.

The present artificial wetland's major consideration was the presence of a second outlet represented by residual buried pipe drains. The mean residence time, variance, and related parameters were determined based on concentrations coming from both surface and pipe drainage outlets considering mixing equations, as written in eq. 17. However, the time to peak concentration referred to the maximum surface outlet concentration value.

*3.2 February 2009: Forest buffer tracer experiment*

The second tracer experiment carried out in the forest buffer alone intended to complement March 2008 tracer experiment results, and provide additional information on longer-term concentration evolution monitoring at the forest buffer outlet. This experiment was the main theme of a master project conducted by Richard (2009).

3.2.1 Chemicals

Six pesticides, three herbicides and three fungicides, were selected for their contrasting properties and wide use in French agriculture (Table III-2). Commercial solutions including the selected molecules were provided by farmers. For being highly concentrated, commercial solutions were diluted before injection.

| Characteristics | Herbicides | | | Fungicides | | |
|---|---|---|---|---|---|---|
| | Glyphosate | Isoproturon | Metazachlor | Azoxystrobin | Cyproconazole | Epoxiconazole |
| Solubility[(a)] (mg/L) | 10500 | 70.2 | 450 | 6.7 | 93 | 7.1 |
| $K_{oc}$ (mL/g) (range) | 21699 (884-60000) | 139 (36-241) | 134 (54-220) | 423 (400-1590) | 390 (173-711) | 1073 (280-2647) |
| log $K_{ow}$ | -3.2 | 2.5 | 2.49 | 2.5 | 3.09 | 3.3 |
| Field $DT_{50}$ (d) (range) | 12 (5–21) | 23 (12–33) | 6.8 (2.8–114) | 21 (3–164) | 36 (28–144) | 120 (44-226) |

**Table III-2: Pesticide main characteristics (FOOTPRINT, 2010). [(a)]Solubility is in water at 20 °C.**

Glyphosate (N-(phosphono-methyl-glycine), GLY) is a broad-spectrum herbicide commonly used in forestry and in agriculture. Glyphosate is a non-mobile pesticide characterized by high $K_{oc}$ values, ranging from 884 (loamy sandy soil) to 60000 mL/g (clay soil) (Table III-2). Glyphosate sorption is mostly influenced by soil clay fraction and Al and Fe oxides and hydroxides contents (Borggaard and Gimsing, 2008). Aminomethylphosphonic acid (AMPA), its main metabolite, was also analyzed. Despite their low mobility, glyphosate and AMPA are frequently detected in surface waters. Isoproturon, presented in section 3.1.1, is a fairly mobile herbicide. Metazachlor (2-chloro-*N*-(pyrazol-1-ylmethyl)acet-2',6'-xylidide, MTZ) is a selective herbicide inhibiting germination, moderately mobile and soluble (Table III-2). It is applied on rape between November and January. Azoxystrobin (methyl(*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate, AZX) is a fungicide inhibiting respiration with curative properties. It is used on vineyards but also on rape in combination with cyproconazole in spring. Cyproconazole ((2RS,3RS)-2-(4-Chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, CYP) is a fungicide providing preventive and curative action, applied on wheat between the end of April and May. Epoxiconazole

((2*RS*,3*SR*)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl) propyl]-1*H*-1,2,4-triazole, EPX) is a systemic fungicide providing preventive and curative action, also applied on wheat between the end of April and May.

### 3.2.2 Tracer experiment

The second forest buffer tracer experiment took place from 19 February 2009 10:50 to 5 March 2009, 13:20. Only one significant rainfall event happened on 4 March 2009 between 6:30 and 12:45 with a cumulative rainfall depth of 9.94 mm. The tracer experiment occurred during the 2008–2009 intense drainage season (Fig. I-12). Temperatures rarely exceeded 9 °C with maximum temperatures close to or greater than monthly averages. The experiment plot surface area was 54 m² (36 m × 1.5 m) (Fig. III-13).

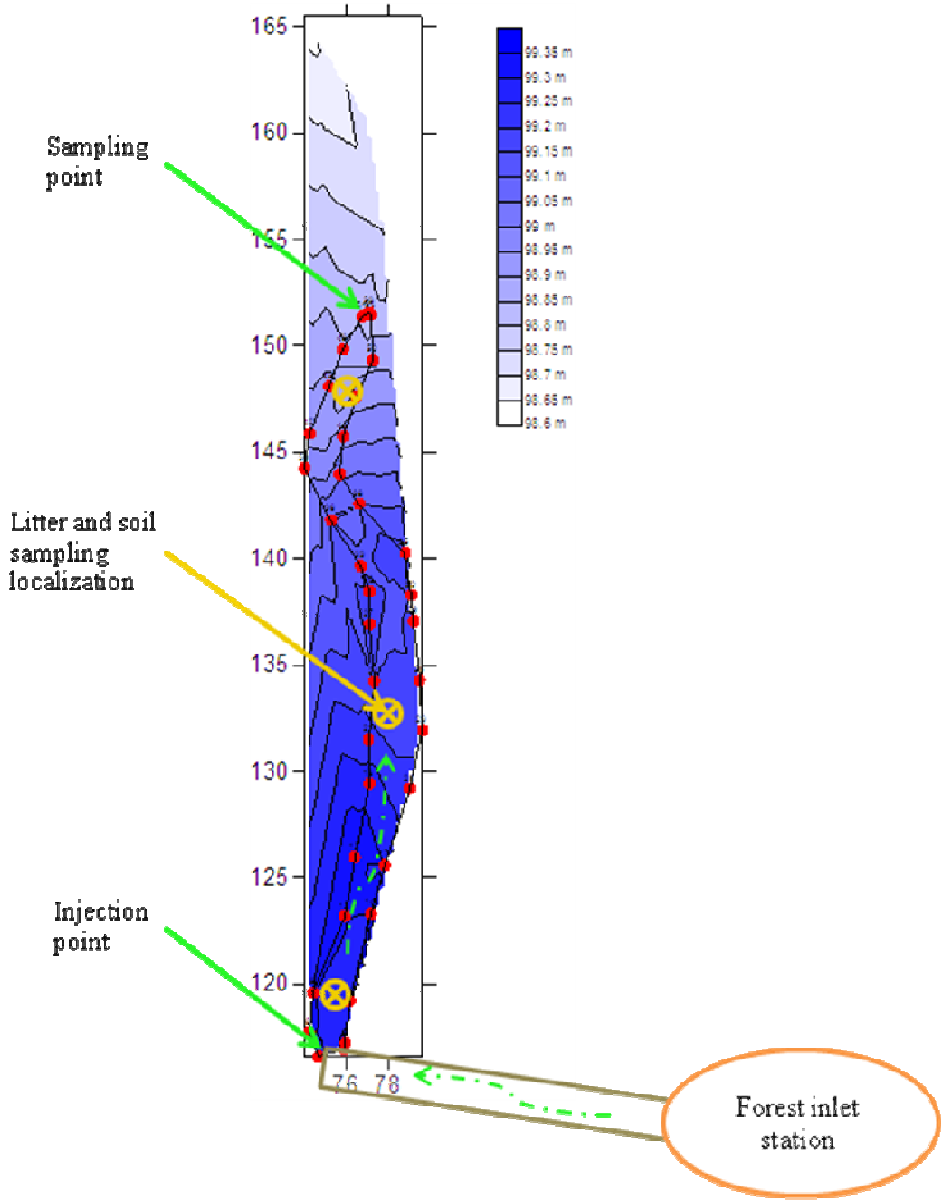



**Fig. III-13: Experimental plot diagram presenting sampling locations. The dotted arrow shows runoff direction. Crossed circles indicate soil and litter sampling points. Topography is represented with contrasted blues (the higher elevation, the darker the blue).**

The inlet flow rate was controlled by reducing the inlet pipe diameter in order to maintain a permanent flow rate at approximately 0.3 L/s (Fig. III-14).

**Fig. III-14: Inlet controlled section to maintain a 0.3-L/s flow rate.**

Soil border levees (Fig. III-15a) limited the experimental plot. At the outlet, a flow restriction (Fig. III-15b) helped manually measuring flow rates by frequently timing the filling of a known volume container. These gaugings were compared to the corresponding inlet flow rates resulting in a 0.59 ratio on average between outlet and inlet flow rates. Outlet flow rates were estimated applying this ratio to continuously measured inlet flow rates for further calculation purposes.


**Fig. III-15: Experimental plot; (a) levees (red arrows) and runoff direction (blue arrow); (b) outlet flow constriction (water color is due to injected uranine). From (Richard, 2009).**

The forest buffer inlet was open on 18 February 2009 at 15:50, in order to saturate the soil and ensure a permanent flow rate for the next day injection. The 22-L injected solution was prepared in a 30-L bucket. The injected solution contained the previously listed six pesticides, potassium bromide (non-reactive water tracer), uranine (dye tracer) and sodium chloride (conductivity tracer). Two peristaltic pumps (Eijkelkamp) were used to ensure a 0.3 L/s injection flow rate during 78 s (pulse injection). Grab water samples or samples collected by means of a time-dependent automated sampler (ISCO 3700, see Fig. I-5) were taken at two locations: (i) at the outlet of the reduced experimental plot and (ii) at the outlet of the whole forest plot. Furthermore, five water samples were also taken at the inlet of the forest in order to monitor pesticide background concentrations during the tracer experiment. Sample collection time step ranged from 30 min to 1 h since the injection time (19 February 2009, 10:50) according to the progress of the dye tracer (uranine). Once uranine reached the outlet of the experimental plot (approximately one hour after the pulse injection), sample collection

time interval was reduced to 15 min for 6 hours. Afterwards, one sample was taken every 30 min during 18.5 hours (from 7 to 25.5 hours after injection). Time intervals were subsequently increased up to 3 hours during the next 73 hours (from 25.5 to 98.5 hours after injection). Finally, sample collection was carried out every 10 hours during the next 240 hours (from 98.5 to 338.5 hours after injection) in order to assess possible pesticide desorption. Water samples were collected in 500-mL amber PET bottles except from 19 February 2009 19:20 to 20 February 09:20, and from 20 February 12:20 to 5 March 13:20, during which an automatic sampler with multi-flasks (330-mL transparent glass tubes) was installed. All samples were stored at 4°C before 4-mL subsamples were extracted and filtered. Subsamples were analyzed for bromide with an ion chromatograph (DX-120, Dionex, Sunnyvale, CA, U.S.A.). Vials were stored at 4°C and PET bottles were frozen until further pesticide analysis was conducted by a subcontracted laboratory (Institut Pasteur de Lille, France). Metazachlor, cyproconazole, epoxiconazole, azoxystrobin, isoproturon and two of its metabolites, desmethylisoproturon and 1-(4-isopropyl phenyl) urea, were extracted by solid-phase extraction (SPE) and then analyzed by high performance liquid chromatography (Agilent 1200) coupled with triple quadrupole mass spectrometry (Micro Mass Ultima or API 4000 Sciex) (LC-MS-MS) with a 0.02 µg/L limit of quantification (LOQ) for each one. Glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) were first derivatized with 9-fluorenylmethyl chloroformate (FMOC) before the LC-MS-MS analysis with a 0.1 µg/L LOQs. ELISA tests were carried out for isoproturon and glyphosate. Isoproturon kits were provided by Envirologix (Portland, ME, U.S.A.) and glyphosate kits by Abraxis (Warminster, PA, U.S.A.). Isoproturon ELISA test is a competitive test where isoproturon competes with isoproturon-enzyme conjugate for a limited number of antibody binding sites on test wells' internal surfaces. Glyphosate samples required a preliminary derivatization before running the assay. Derivatized samples were subsequently added in each well of the kit plate with glyphosate antibody solution and glyphosate enzyme conjugate. Each plate contained 96 wells and was read at 450 nm wavelength with an absorbance plate reader equipped with a tungsten halogen (ELx800, BioTek, Winooski, VT, U.S.A.) and the software Gen5™.

### 3.2.3 Litter & soil sampling and analysis

Litter and soil grab samples were taken at the end of the tracer experiment at the forest plot inlet, middle and outlet. Another one was taken outside the experimental plot in the forest buffer. All samples were frozen before pesticide analysis by the Institut Pasteur de Lille. Glyphosate and AMPA were extracted by ultrasonic waves in water, then derivatized with FMOC and analyzed by LC-MS-MS, whereas the other molecules extraction was carried out with ultrasonic waves in acetone for soil samples. Extracts were analyzed by LC-MS-MS. Litter samples were treated with an internal procedure developed by the Institut Pasteur de Lille. Limits of quantification were 0.01 mg/kg dry matter for each compound.

### 3.2.4 Data analyzes

The Manning-Strickler equation was used in order to estimate the water level into the experimental plot:

$$Q_{out} = K * S * R^{2/3} * i^{1/2} \quad \text{(Eq. 25)}$$

where $Q_{out}$ is estimated outlet flow rate ($m^3/s$) average, $K$ is the Strickler roughness coefficient ($m^{1/3}/s$), $S$ is the cross sectional area, $R$ is the hydraulic radius (m) and $i$ is the surface profile slope (m/m).

Loads were calculated as follows:

$$m_{out}(t_i) = \sum_{1}^{i} C_{out}(t_i) \times \frac{Q_{out}(t_i) + Q_{out}(t_{i-1})}{2} \times (t_i - t_{i-1}) \quad \text{(Eq. 26)}$$

where $m_{out}(t_i)$ is the outlet cumulated mass of a pesticide at time $t_i$ after injection, $C_{out}$ its concentration (µg/L). Concentrations lower than the limits of quantification (LOQ) were set to the LOQ divided by 5. No difference was provided by the laboratory for detection and quantification thresholds. Recovery rates were then calculated as shown in equation 2 for the first 24 hours after injection and called "$R_{recovery}$" as well as the subsequent 13 days and referred to as "$R_{released}$". In order to estimate possible occurrence of other processes than dilution alone, maximal to initial outlet concentration ratios of bromide were compared with those of pesticides. For higher bromide than pesticide ratios, other processes than dilution can be suspected to influence pesticide transfer. Three grab water samples were taken at forest buffer inlet to control pesticides' background concentrations coming from the artificially drained watershed. Outlet concentrations were adjusted accordingly when pesticides were detected at the inlet by subtracting inlet to outlet concentrations (negative differences were assumed to be null).

Bromide was detected the first 24 hours. Adimensional concentrations were calculated by dividing outlet concentrations by maximal concentration (peak) thus helping graph comparisons among pesticides and bromide tracer. To easily compare pesticide concentrations to those of bromide, the results' analysis was divided into two distinct periods: (i) a period during which sorption was expected, starting from the injection time to that when all injected bromide had passed through the plot (first 24 h), and (ii) a period where desorption was expected, from the latter time to the end of the tracer experiment.

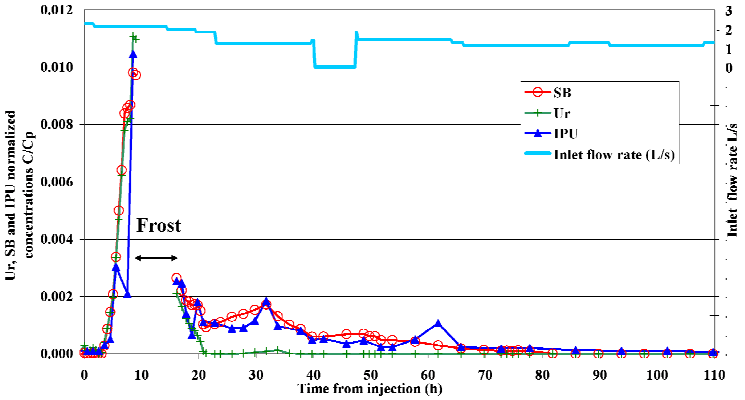
## 4 Results and discussion

### *4.1 March 2008 experiment: internal hydraulics and tracer transfer characterization*

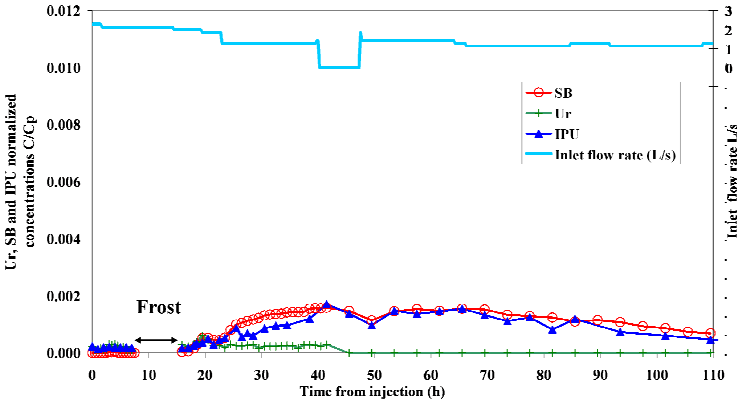
Once in the buffer zones, pesticide concentration reduction can be expected provided there is a sufficient contact time for pesticide adsorption or degradation to occur. The hydraulic retention time is a key parameter to estimate water storage duration within the systems. The multi-tracer experiment was conducted under nearly steady-state conditions with an average flow rate of approximately 1.36 ± 0.47 L/s at the wetland inlet and outlet. The average flow rate was lower (0.20 L/s) for the forest. This is without considering the large rainfall event that occurred at the end of the experiment on 10 March 2008. It is important to note that the results of the on-site experiment depend on actual flow conditions. The low-flow conditions are unusual in early March in this area.

#### 4.1.1 Tracer dynamics

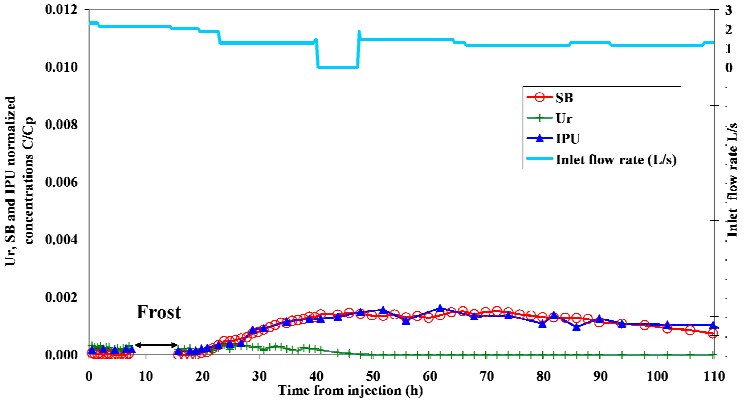
Fig. III-16 and Fig. III-17 provide dye tracer response curves. Breakthrough curves were asymmetric, presenting a right-skewed distribution departing from ideal plug flow conditions.



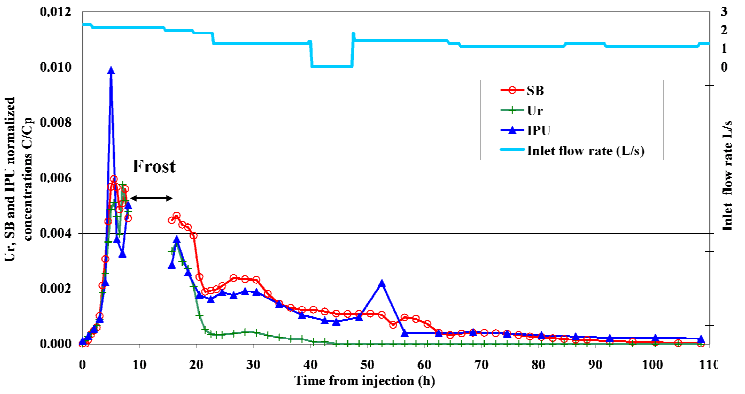

(a)




(b)




(c)




(d)

**Fig. III-16: Tracer concentration dynamics at the different wetland outlets (SB, sulforhodamine B ; Ur, uranine ; IPU, isoproturon). (a), (b) and (c) were concentration at the surface outlet of AW1, AW2 and AW3, respectively, over time. (d) Concentration at the outlet of the pipe drainage located under the artificial wetlands over time.**

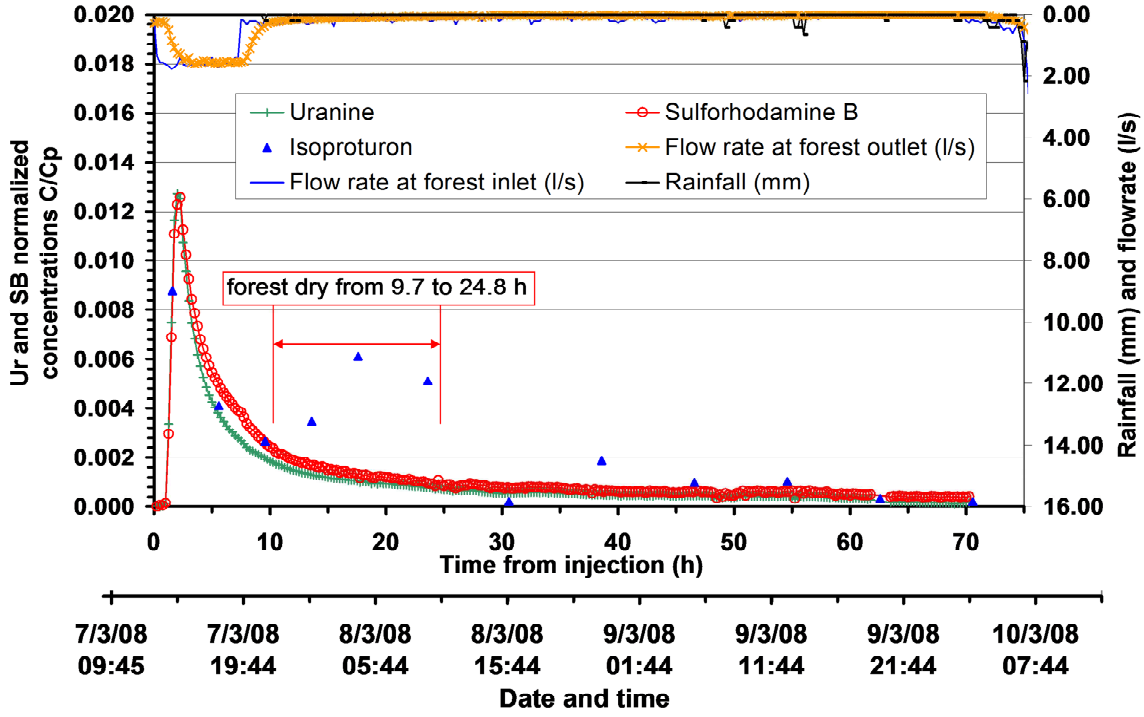



**Fig. III-17: Tracer concentration dynamics at the forest outlet (SB, sulforhodamine B ; Ur, uranine ; IPU, isoproturon).**

### *4.1.1.a Peak concentrations*

Tracer concentration peak reduction was observed, indicating dilution having occurred between inlet and outlet of the systems. At the forest buffer outlet, uranine and sulforhodamine B concentration peaks were observed 2.5 h after tracer injection after 12.5 $m^3$ of water had passed through. Despite a small recovery rate (31 %), a 5.13 h residence time was calculated based on sulforhodamine B values. Forest travel time may be expected to be shorter for wetter initial soil conditions when infiltration is reduced. This is generally observed for grassed buffer zones (Muñoz-Carpena et al., 1999). However, the experiment was only conducted on a small part of the forest buffer zone. A wider area would be available for water to run over during regular performance of the forest buffer zone. This might increase the time needed for concentration peaks to reach the forest outlet.

The artificial wetland surface outlet (AW3) was reached by a non-significant uranine peak concentration 19 h after injection (after 137.2 $m^3$ of water had passed through the outlet), whereas sulforhodamine B and isoproturon peak concentrations were observed in 62 h, after 320.7 $m^3$ had passed through the outlet.

### *4.1.1.b Uranine*

After reaching AW2, uranine peaks disappeared nearly totally, most probably because of photo-decay effect. The decrease in uranine concentration was less pronounced in the forest buffer and along the pipe drainage travel path of the artificial wetland than along the surface travel path of the artificial wetland.

| | Three-cells artificial wetlands | | | Forest buffer | | |
|---|---|---|---|---|---|---|
| | Ur | SB | IPU | Ur | SB | IPU |
| Recovery rate % | 19 | 87 | 70 | 43 | 31 | 21 |

**Table III-3: Tracer recovery rates for uranine (Ur), sulforhodamine (SB) and isoproturon (IPU). .**

At the forest buffer outlet, uranine recovery rate was larger than that at the AW outlet but apparent losses remained. As uranine is only slightly sorptive (Käss, 1994), forest losses could be attributed to photodegradation and tracer losses by infiltration and side-leaks out of the limited study area.

#### *4.1.1.c Sulforhodamine B*

In the artificial wetlands, few losses were obtained for sulforhodamine B presenting an 87 % recovery rate, whereas only 31 % of total injected mass was recovered at the forest buffer outlet (Table III-3). Sulforhodamine B was considered the most conservative tracer in this study for AW and was therefore used for further calculations.

#### *4.1.1.d Isoproturon*

Similar recovery rates than sulforhodamine B were found for isoproturon with 70 % in the artificial wetland (*STR* = 0.09 %/m$^3$) and 21 % (*ATR* = 0.15 %/m²) in the forest buffer. In the artificial wetland, isoproturon *ATR* (0.02 %/m²) and *STR* (0.09 %/m$^3$) values were very similar to those found by Kidmose et al. (2010). These authors observed 66 % of injected isoproturon removal leading to *ATR* and *STR* values of 0.02 %/m² and 0.03 %/m$^3$, respectively. The forest *ATR* value seems to indicate that, on an areal-basis, forest buffers could present a higher potential than artificial wetlands to reduce pesticide pollution.

#### *4.1.1.e Discussion*

As the forest buffer had more shade, this argues for uranine being more likely photo-decayed in AW than in FB. Consequently, all other things being equal, light-sensitive molecules, i.e., certain applied herbicides such as bromoxynil, may be susceptible to degradation to a greater extent in the AW than in the FB. Despite late-day injection, uranine recovery rates were very low in the AWs, as expected since this constituent was highly photodegradable (Smart and Laidlaw, 1977) (Table III-3).

Wetland low plant density may have prevented extensive adsorption. On the other hand, in the forest buffer, water ran off through the litter layer enriched in organic matter (Benoit et al., 2008). This suggests more important preferential flows in macropores or a possible greater adsorption of sulforhodamine B or isoproturon in the forest buffer than in the artificial wetlands. Consequently, it appeared that the forest buffer might be more effective at reducing pesticides than the full artificial wetlands. Since isoproturon and sulforhodamine B have similar sorbing properties, the difference in their recovery rates may be due to isoproturon degradation because sulforhodamine B is a more stable molecule. However, isoproturon metabolites were not analyzed. Consequently, even when full, the artificial wetland is likely to provide some dissipation even for mobile molecules such as isoproturon. It is important to note that these results are valid for steady-state flow conditions. Forest buffer performance may decrease considerably during large floods. Indeed, the forest soil is likely to reach saturation, thus reducing infiltration and contact time between pollutants and potential adsorbing sites, as observed for some vegetative buffer zones (Souiller et al., 2002). Slightly mobile pesticides (e.g., isoproturon and chlorotoluron) with low to moderate adsorption properties are thought to be reduced more easily in the forest buffer than in sparsely planted artificial wetlands. In spring, fall, and summer, more vegetation is present than in winter. In addition, it was found that when the difference between precipitation and evapotranspiration is minimum (e.g., in spring and summer), higher load reduction can be expected (Borin et al., 2004).

It should be pointed out that at the outlet of the remaining buried drainage located under the artificial wetland, concentration peaks were slightly reduced and appeared very quickly after injection. The remaining pipe drainage provided the advantage of helping empty the wetlands. However, it also behaved like short-circuits in that it created the first peaks prior

to surface outlet peaks for each artificial wetland. The first peak at the pipe drainage outlet (5.5 h after injection) supported the idea of the presence of pipe drains or pipe drain holes under AW1. The remaining pipe drains were not deep, leaving few opportunities for tracers or pollutants to sorb onto soil particles. This is why remaining drains were blocked from 03 Dec. 2008.

*4.1.1.f Flow-weighted composite sample concentrations*

Isoproturon concentrations in flow-weighted composite samples taken after the end of the tracer experiment were 3.02 ± 0.17 (from 10 to 16 March 2008), 0.43 ± 0.03 (16 to 23 March 2008) and 0.59 ± 0.04 µg/L (23 March to 6 April 2008) at the artificial wetland outlet. A misplacement of the single bottle in forest outlet automated sampler prevented from getting a water sample right after the tracer experiment. A concentration of 30.33 ± 2.01 µg/L was measured in the next sample (16 to 23 March 2008) after which the forest was closed and no more sample collected. These results show that some isoproturon was still slowly transferred through, or desorbed from, the buffer zones.

4.1.2 Hydrology performance assessment

The whole wetland (the three cells in series) had a mean retention time of 66.5 h according to sulforhodamine B data. Table III-4 shows additional metrics used to assess the artificial wetland hydrology performance.

| | L/W | $\tau$ h | $T_n$ h | e | $\sigma^2$ h² | $\sigma^2_\theta$ | $t_p$ h | N | $\lambda$ |
|---|---|---|---|---|---|---|---|---|---|
| AW1 | 5:1 | 18.6 | 14.9 | 1.25 | 231.7 | 0.67 | 9.0 | 1.5 | 0.42 |
| AW1 + AW2 | 5:1 | 64.9 | 51.3 | 1.27 | 1591.6 | 0.38 | 42.0 | 2.7 | 0.79 |
| 3-cells AW | 20:1 | 66.5 | 76.2 | 0.87 | 1634.0 | 0.37 | 62.2 | 2.7 | 0.55 |

**Table III-4: Estimated wetland hydraulic metrics from sulforhodamine B data for an inlet flow rate of 1.37 L/s. L, wetland length; W, wetland width; $\tau$, mean residence time; $T_n$, nominal residence time; *e*, effective volume ratio; $\sigma^2$, variance; $\sigma^2_\theta$, normalized variance; $t_p$, time to peak; N, number of tanks in series; $\lambda$, hydraulic efficiency.**

Nominal residence times ($T_n$) for AW1 (14.9 h) and AW1+AW2 (51.3 h) were smaller than the mean residence times (18.6 h and 64.9 h, respectively) leading to effective volume ratios greater than unity (Table III-4). This may be understood as an indication of the tracer retention and further remobilization in AW1 and AW2. In a previous study (Borin et al., 2004), a $T_n$ smaller than $\tau$ was also found for two subsurface horizontal flow beds treating wastewaters. When focusing on the whole wetland, the effective volume ratio was smaller than unity, indicating that 87% (e = 0.87) of the three-cell artificial wetland volume was effectively used, suggesting few dead zones and confirming observations of such stagnant areas in some corners in AW1 and AW2. The present tracer experiment was conducted in winter. Frost occurred during the first night, likely creating water temperature stratification in the artificial wetland. This phenomenon may have caused effective volume extension by generating water movements (Torres et al., 1997). Under these specific conditions, a 66.5-h mean residence time may provide some pesticide reduction, particularly for only slightly mobile, sorbing, or photo-sensitive molecules. However, isoproturon decrease was not extremely high under 66.5-h residence time and potentially subjected to further release. This residence time value may therefore not be sufficient to ensure proper degradation of pesticide molecule having moderate to high degradation half-lives. It is important to note that for larger flow rates, e.g., during floods, the mean retention time would be reduced affecting wetland pesticide reduction efficiency. On the other hand, vegetation coverage became significantly denser throughout the 2007 – 2010 monitoring period which may have increased residence

time under similar inflow conditions. The increase in variance indicates an increasing degree of mixing in the wetland. For pesticide non-point source pollution mitigation, this may be desired because it enables dilution of highly concentrated influents. However, when assuming first-order kinetics for pollutant removal, it can be easily demonstrated that higher removal rates are provided for plug flow ($\sigma^2_\theta = 0$) than mixed ($\sigma^2_\theta > 0$) systems (Kadlec and Knight, 1996). Although a first-order reaction is often assumed for pesticide reduction in wetlands (Rodgers and Dunn, 1992; Moore et al., 2001b), this may not be the most consistent model (Kadlec, 2000) to use. For the three-cell artificial wetland of this study (L/W = 20:1), the dimensionless variance was 0.37, indicating moderate mixing theoretically associated with 2.7 continuously stirred tank reactors. Jenkins and Greenway (2005) found a much lower variance for a non-vegetated wetland model (0.0326 for L/W = 17.5). They also demonstrated that increasing fringing vegetation density or cover implied an increase in the degree of mixing (from 0.0609 to 0.496 for 20.0 to 70.0 % vegetation cover, respectively) but lowered wetland hydraulic efficiency at the same time (from 0.888 to 0.351). This is due to the fact that fringing vegetation created short-circuiting in the central non-vegetated zone of the wetland. However, they also demonstrated that banded vegetated wetlands presented slightly greater variances and hydraulic efficiencies than similarly shaped non-vegetated wetlands. Holland et al., (2004) found dimensionless variances of 0.23 and 0.41 for low and high water level experiments, respectively.

In the present study, the first two wetlands cells had hydraulic efficiency of 0.79. AW1 and AW2 both included one berm to force circulating water and limit short-circuits. On a study based on 13 virtual ponds with no vegetation, Persson (2000) compared different designs and associated hydraulic efficiencies ($\lambda$). The overall hydraulic efficiency of 0.79 was close to that found by Persson et al. (1999) for a pond including three baffles for which $\lambda$ was 0.76. According to Persson et al.'s (1999) classification, the 0.79 value that we found can be considered as a good hydraulic efficiency, whereas 0.55 (the whole system's hydraulic efficiency) is satisfactory and 0.42 (AW1) is poor. After rejuvenating a 490-ha wetland, Wang and Jawitz (2006) showed increased average hydraulic efficiency from 0.34 to 0.74. Rejuvenating mainly consisted of plant and sediment removal, site grading within the wetland cells, baffle and island construction, and re-vegetation. The value found for the Bray artificial wetlands is therefore within the range of those found in previous studies.

### *4.2 February 2009: Forest tracer experiment*

#### 4.2.1 Hydrology

In the second forest tracer experiment, the estimated outlet flow rate averaged 0.18 L/s. Using a 25 $s/m^3$ K coefficient as usually done for vegetated ditches in the Manning-Strickler equation, an average water level of 2.34 mm was calculated. This value is in concordance with on-site sheet flow observation. Such a shallow sheet flow guaranteed optimal surface contact between forest soil and litter and pesticides which is crucial to enhance pesticide retention (Margoum et al., 2003). As shown in Chapter II, adsorption is expected on these two substrates even if it may be reversible particularly from organic vegetal substrates like forest litter. Bromide started to be detected one hour after injection and reached a peak 1.75 h after injection. Bromide concentrations were below detection limits 24 h after the start of the tracer experiment (Fig. III-18).

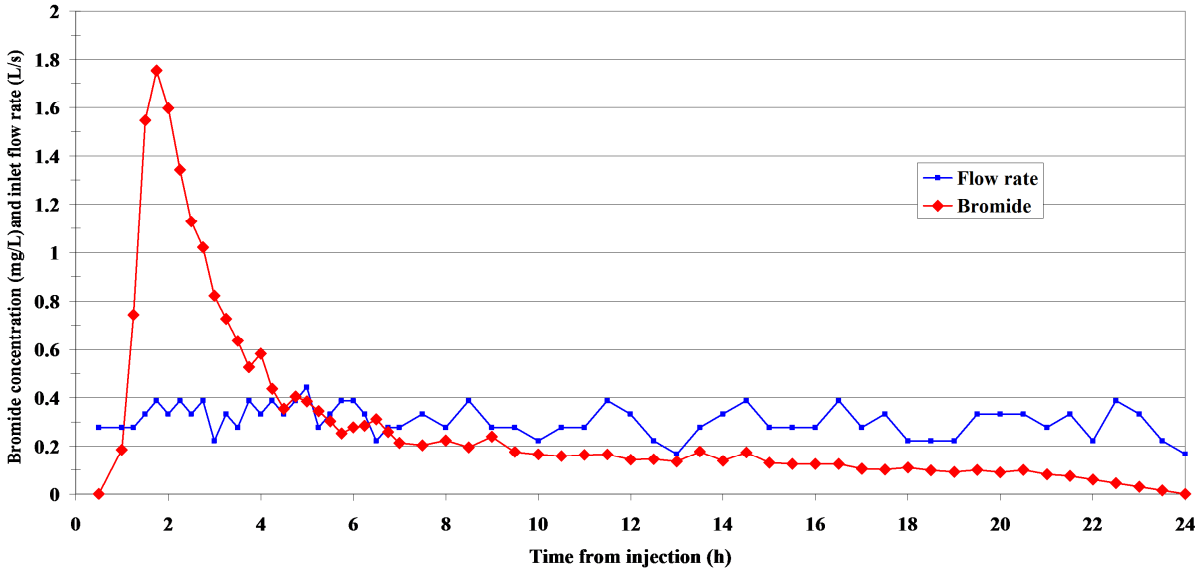



**Fig. III-18: Bromide concentration (red) and flow rate (blue) at the forest plot outlet.**

Bromide recovery rate was 73.5 % and hydraulic residence time was 6.32 h. This allowed a non-negligible contact time between pesticides in solution and the litter. This value is fairly similar to that estimated during March 2008 tracer experiment (5.13 h) although low recovery rates were found. Tracer injection was carried out at the forest experimental plot inlet, located approximately 25 m further down the forest buffer inlet where was situated the electromagnetic flowmeter. Water losses may have taken place in this 25-m long inlet ditch. In addition, some water leaks were observed out of the experimental plot although delimited with levees that were reduced as much as possible. In addition, subsurface flow short-circuits may have been created by low soil levee compaction, earthworm burrows or tree roots.

### 4.2.2 Inlet water quality

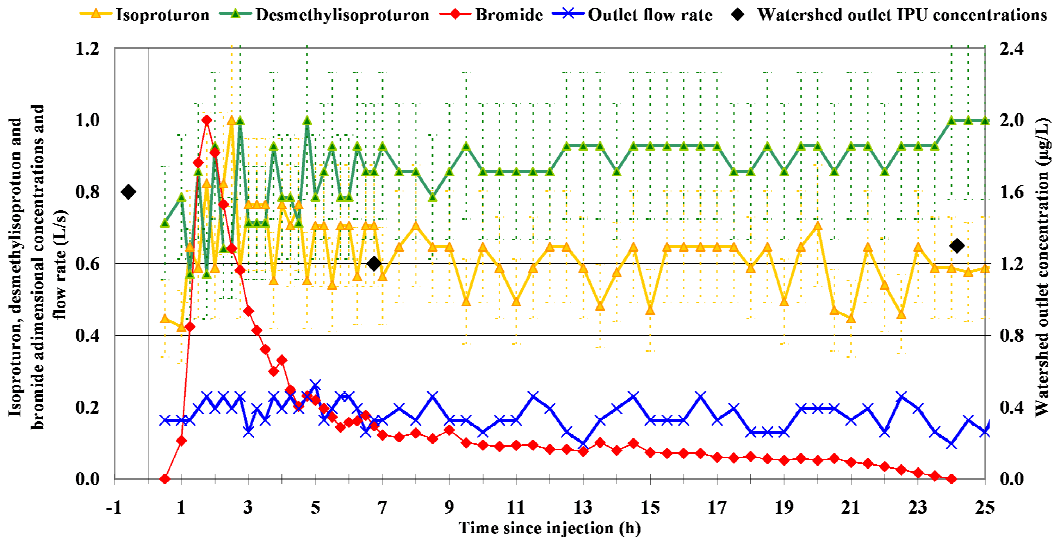
Five grab water samples taken at the forest inlet (i.e. watershed outlet) showed non-negligible concentrations of isoproturon, desmethylisoproturon, glyphosate, AMPA and metazachlor (Table III-5). Epoxiconazole was detected once (19 Feb. 2009 17:35) but with a concentration lower than the limit of quantification. Previous applications of glyphosate (18 Oct. 2007) and metazachlor (3 Sept. 2007) were approximately 16 months before the tracer experiment. That of isoproturon, dating from 23 Dec. 2008, only 2 months earlier, may explain the measured high concentrations (> 1.2 µg/L except on 23 Feb. 2009, Table III-5). It resulted in additional 169 mg of isoproturon entering the experimental plot with watershed outlet flows. This is much larger than isoproturon injected load (3.59 mg) for the tracer experiment (Table III-5). High uncertainties on outlet concentrations interpretations are therefore to be expected. Corrections were attempted on forest experimental plot outlet concentrations to account for these values.

| Sampling time | AMPA[(a)] | Isoproturon[(b)] | Desmethylisoproturon[(b)] | Metazachlor[(b)] |
|---|---|---|---|---|
| 19/02/2009 10:15 | 0.30 | 1.60 | 0.12 | 0.29 |
| 19/02/2009 17:35 | x[(c)] | 1.20 | 0.11 | 0.30 |
| 20/02/2009 11:00 | n.d. | 1.30 | 0.10 | 0.25 |
| 23/02/2009 14:10 | 0.30 | 0.27 | 0.03 | 0.11 |
| 05/03/2009 13:50 | 0.30 | 1.40 | 0.11 | 0.19 |

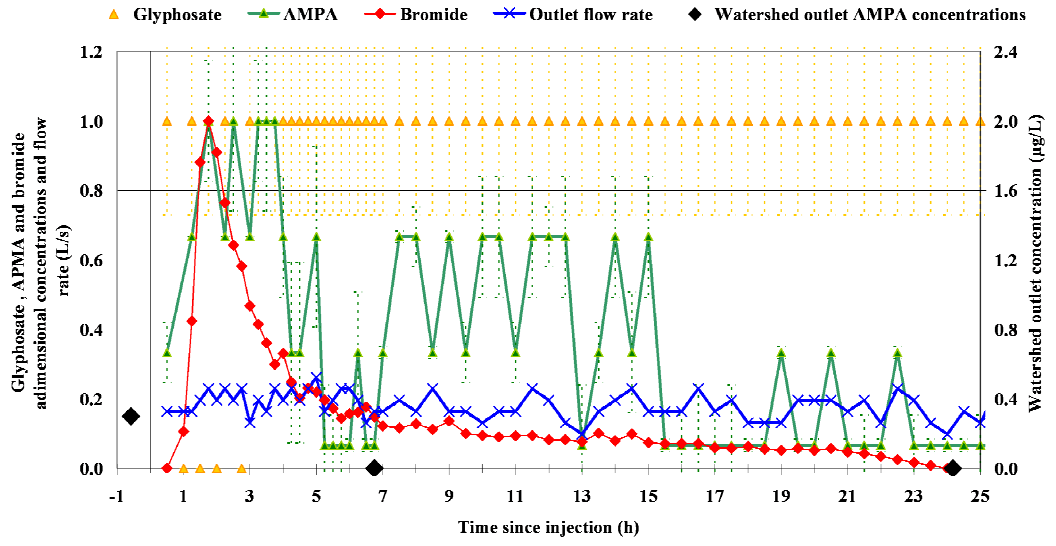
**Table III-5: Forest buffer inlet grab water sample concentrations. [(a)]limit of quantification (LOQ) is 0.1 µg/L ; [(b)]LOQ is 0.02 µg/L. [(c)]not analyzed for AMPA. n.d. is non detected.**

## *4.3 Dissipation period: first 24 hours*

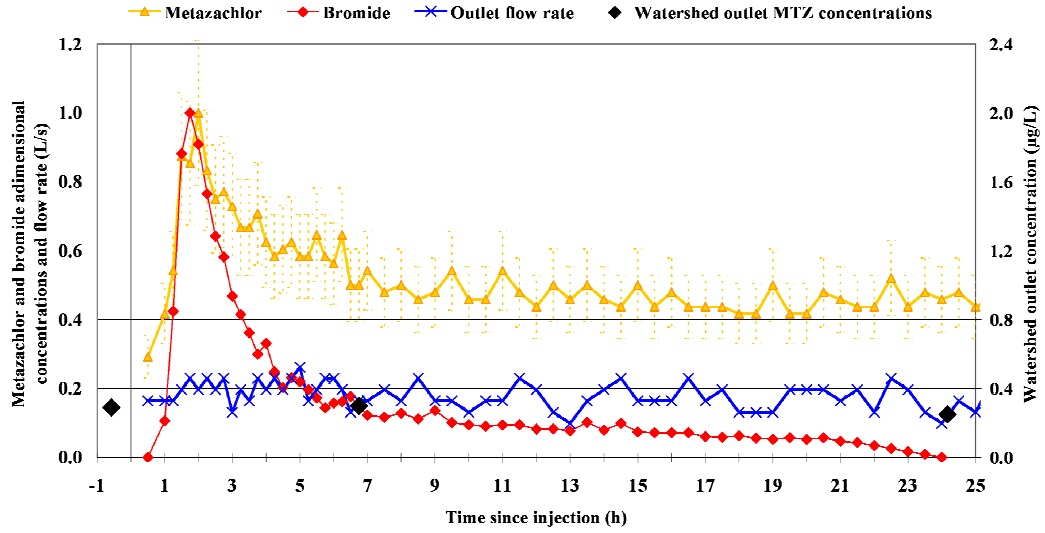
### *4.3.1.a Pesticide dynamics description*



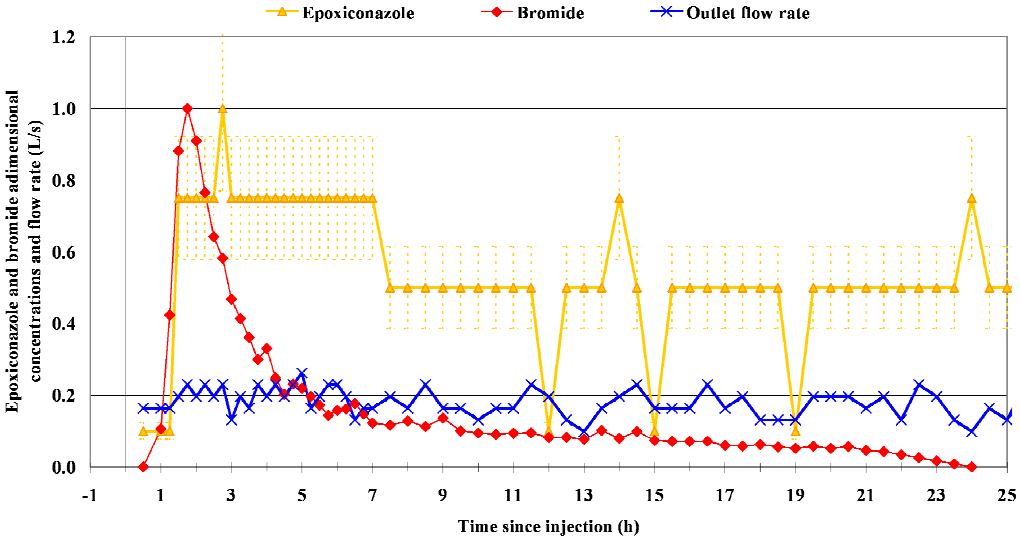

(a)



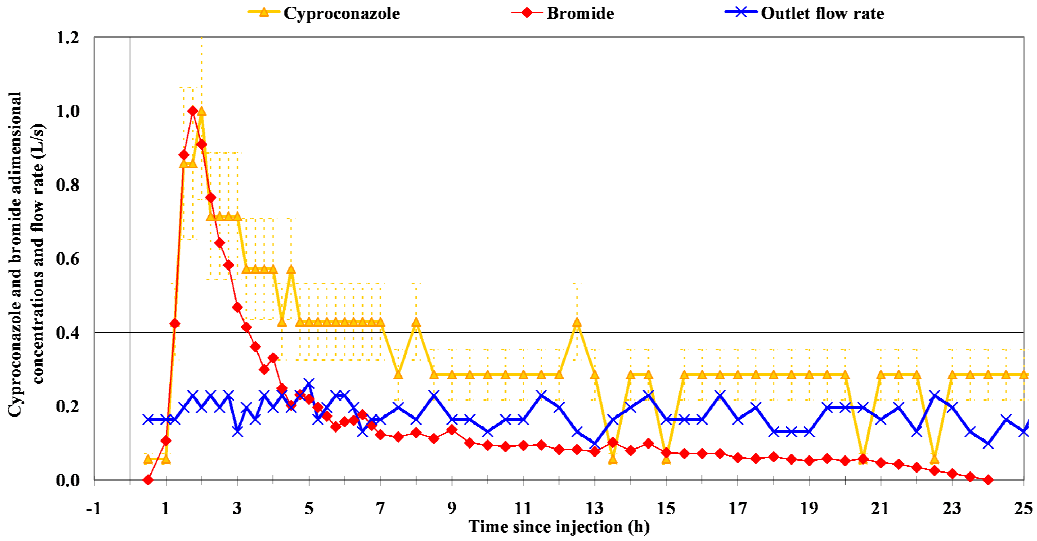

(b)


Metazachlor
Bromide
Outlet flow rate
Watershed outlet MTZ concentrations
Metazachlor and bromide adimensional concentrations and flow rate (L/s)
Watershed outlet concentration (µg/L)
Time since injection (h)

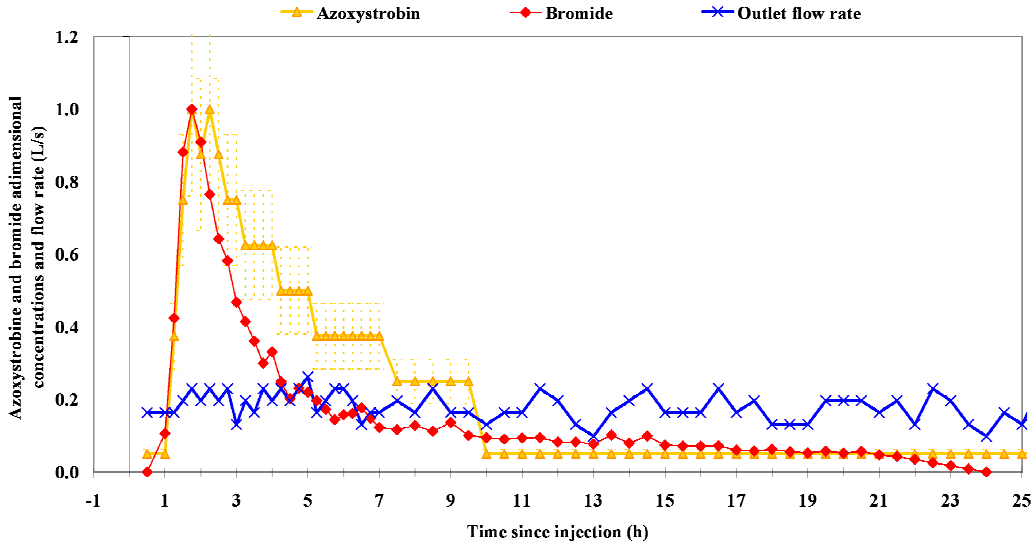
(c)


Epoxiconazole
Bromide
Outlet flow rate
Epoxiconazole and bromide adimensional concentrations and flow rate (L/s)
Time since injection (h)

(d)


Cyproconazole
Bromide
Outlet flow rate
Cyproconazole and bromide adimensional concentrations and flow rate (L/s)
Time since injection (h)

(e)




(f)

**Fig. III-19: Forest experimental plot outlet dynamics for pesticide parent molecules (yellow), metabolite (green), bromide (red) and flow rate (blue) for the first 24 h after injection. Concentrations are normalized to maximal concentration values. ♦ are watershed outlet concentrations in grab water samples. Rain was null during this period. (a) Isoproturon and desmethylisoproturon, (b) Glyphosate and AMPA, (c) Metazachlor, (d) Epoxiconazole, (e) Cyproconazole and (f) Azoxystrobin.**

Pesticide concentrations at the experimental plot outlet for the first 24 hours after injection are presented in Fig. III-19. Table III-6 presents tracer experiment main dynamics and mass balances characteristics. Apart from isoproturon presenting fairly high concentrations (on average between 0.8 and 1.3 µg/L), concentrations were lower than 0.5 µg/L for AMPA and metazachlor and did not exceed 0.15 for the other pesticides (azoxystrobin, epoxiconazole, cyproconazole and desmethylisoproturon).

| | $Br^-$ | IPU | MTZ | AZX | EPX | CYP | *GLY* |
|---|---|---|---|---|---|---|---|
| Initial mass (µg)[(a)] | 5860 | 4100 | 2665 | 1107 | 1353 | 574 | 3690 |
| Cinj (µg/L) | 5.72[(b)] | 200 | 130 | 54 | 66 | 28 | 180 |
| First detection time (h) | 1.00 | 1.25 | 1.25 | 1.25 | 1.50 | 1.25 | 178.50 |
| Concentration peak time (h) | 1.75 | 2.50 | 2.00 | 2.00 | 2.75 | 2.00 | 178.50 |
| Concentration peak (µg/L)[(a)] | 1.75 | 0.72 | 0.27 | 0.08 | 0.04 | 0.07 | 0.50 |
| $R_{peak}$ (%) | 0.61 | 0.36 | 0.20 | 0.15 | 0.06 | 0.25 | 0.28 |
| Cumulated outlet mass (µg)[(a)] | 4305 | 1695 | 579 | 270 | 326 | 367 | 302 |
| $R_{recovery}$ first 24 h (%) | 74 | 41 | 22 | 24 | 24 | 64 | |
| *ATR* (%/m²)[(c)] | 0.48 | 1.09 | 1.44 | 1.41 | 1.41 | 0.67 | |
| $R_{recovery}/R_{recovery}$ $Br^-$ first 24 h (%) | 100 | 56 | 30 | 33 | 33 | 87 | |
| $R_{released}/R_{recovery}$ $Br^-$ (24 h to 340 h) (%) | 0 | 16 | 34 | 18 | 31 | 77 | |

**Table III-6: Tracer experiment dynamic characteristics and mass recovery rates. [(a)]Bromide mass and concentration were in mg and mg/L, respectively. [(b)]Bromide concentration is in mg/L. [(c)]*ATR* are area normalized tracer reduction rates. Cinj is concentration in injected solution.**

Despite presenting a very high sorption coefficient and low half-life (Table III-2), glyphosate is frequently detected in surface waters (IFEN, 2007a). Surprisingly, glyphosate

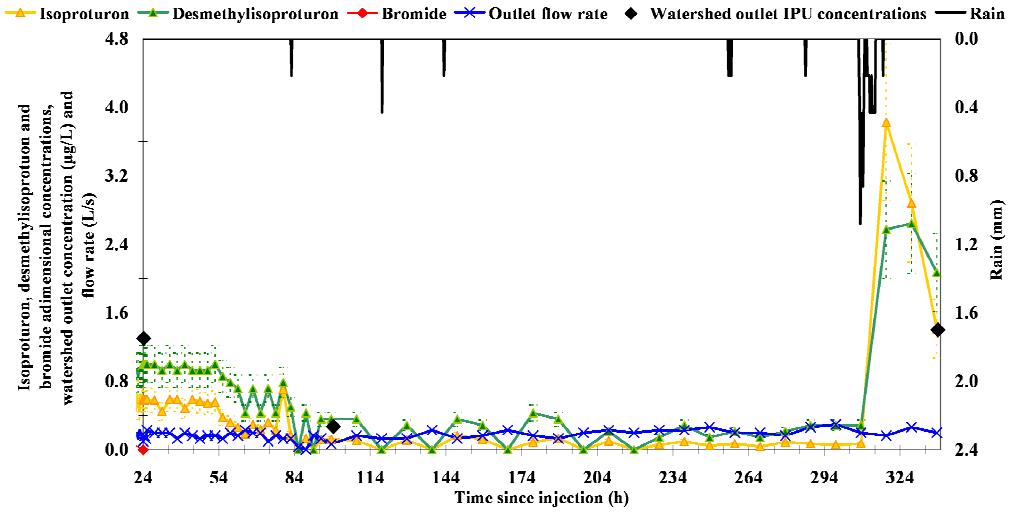
was never quantified in water, soil or litter samples (Table III-7 and Fig. III-19). However, its limit of quantification was high (0.1 µg/L) and no difference was provided between detection and quantification limits.

| Sampling zone | Sample nature | IPU | MTZ | GLY | AMPA | EPX | AZX | CYP |
|---|---|---|---|---|---|---|---|---|
| Inlet zone[(a)] | Litter | 0.01 | n.d.[(c)] | n.d. | n.d. | 0.01 | n.d. | n.d. |
| | Soil | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Middle zone[(a)] | Litter | 0.01 | n.d. | n.d. | n.d. | 0.01 | n.d. | n.d. |
| | Soil | 0.01 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Outlet zone[(a)] | Litter | 0.01 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| | Soil | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Outside expe plot[(b)] | Litter | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| | Soil | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

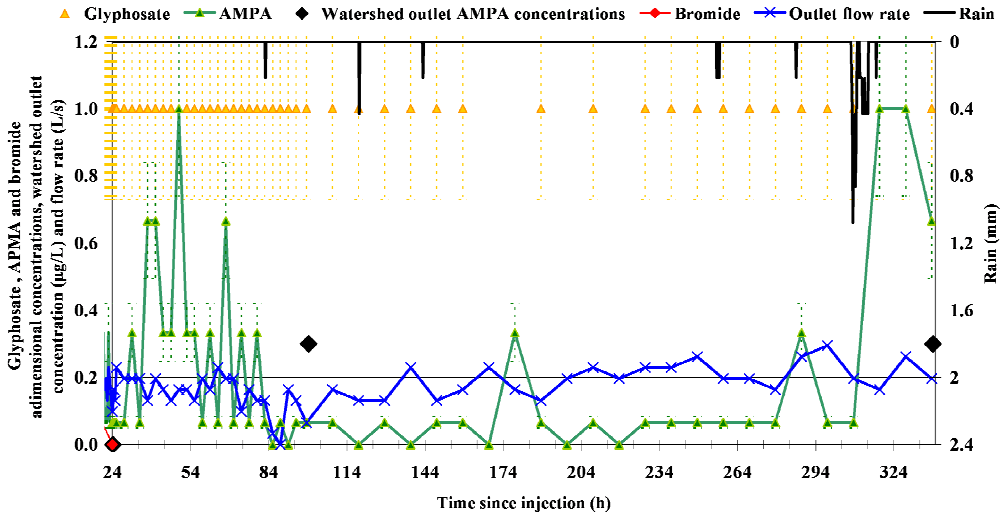
**Table III-7: Pesticide concentrations (mg/kg) in forest litter and 0 – 3 cm soil, in samples taken [(a)]inside or [(b)]outside the experimental plot. [(c)]n.d. are non detected.**

For further calculation purposes, all values set to "lower than the limit of quantification" were replaced by LOQ/5 which may have generated either over- or under-estimations of glyphosate outlet loads. Concentration results resolution was 0.01 µg/L. Consequently, no intermediate value was given between 0.05 and 0.06 µg/L, for instance, leading to graphs showing horizontal levels for cyproconazole, azoxystrobine and epoxiconazole (Fig. III-19d to Fig. III-19f). It is important to note that uncertainties only accounted for laboratory internal reproductibility and ranged from 21 (metazachlor) to 27 % (glyphosate). From Fig. III-19d to Fig. III-19f, it appears that for horizontal levels, uncertainties overlapped from one level to another. This shows that concentration curves would have been smoothened provided a higher resolution on concentration results had been available. Knowing the presence of such uncertainties, it can however be concluded that pesticides were detected and showed a peak slightly after that of bromide. Pesticides' peaks were observed at approximately 2.0 (metazachlor, azoxystrobin and cyproconazole), 2.5 (isoproturon) and 2.75 h (epoxiconazole) after the tracer experiment started (Table III-6) and slowly decreased afterwards (Fig. III-19). Such low differences among pesticide peak detection times can hardly be attributed to pesticide sorbing properties alone. Mass balances were also affected by uncertainties. Their interpretation should therefore be done cautiously. Pesticide recovery rates normalized to that of bromide ranged from 30 (metazachlor) to 87 % (cyproconazole) for the first 24 hours. Glyphosate was only detected once during the tracer experiment (178.5 h after injection). Glyphosate load calculations were only based on LOQ/5 estimated concentration values for the first 24 hours and were likely to present extremely high uncertainties. Its recovery rate was not provided. Recovery rates for the other pesticides were not in accordance with molecule sorption properties, confirming that $K_{oc}$ values should not be the sole explanatory parameter to describe pesticide fate. Metazachlor, azoxystrobin and epoxiconazole showed similar recovery rates (approximately 30 % or 1.40 %/m² *ATR*) despite having different sorption coefficients (Table III-2). Pesticide molecules present a wide range of water or organic solvent solubility, sorbing and degradation properties that could explain their fate in the environment. All areal-normalized reductions (*ATR*) were high. Isoproturon *ATR* value was 0.15 %/m² for March 2008 tracer experiment, whereas it was 1.09 %/m² in

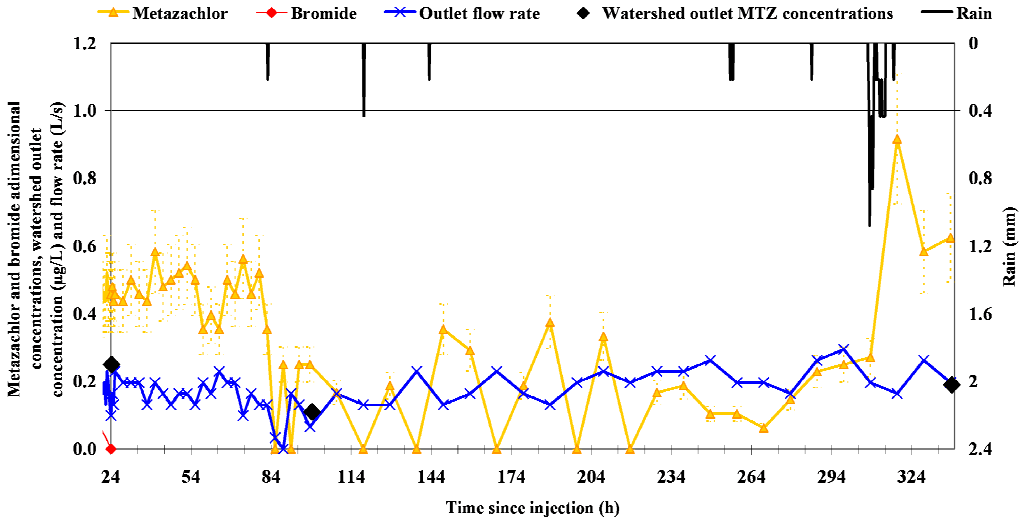
March 2009 tracer experiment. Because of shallower flows in the forest buffer than in the artificial wetland, pesticides and forest substrates' interactions are higher than artificial wetland substrates and pesticides' interactions. However, it is important to highlight that forest buffer water storage capacity is highly reduced compared to that of wetland systems. Forest can not accommodate as large water volumes as artificial wetlands. This should also be kept in mind while comparing performances between different systems. From 24 to 340 h following the start of the tracer experiment, no more bromide was detected. Contrarily, isoproturon, desmethylisoproturon, AMPA and metazachlor were still transferred up to 84 h, and epoxiconazole and cyproconazole were detected up to 54 h (Fig. III-20). Azoxystrobin was not above the limit of quantification after 10 h after injection. It is the pesticide that showed the fastest concentration decrease after peak observation which may be explained by a fairly low injected concentration (54 µg/L) and high adsorption coefficient ($K_{oc}$ = 482 mL/g).



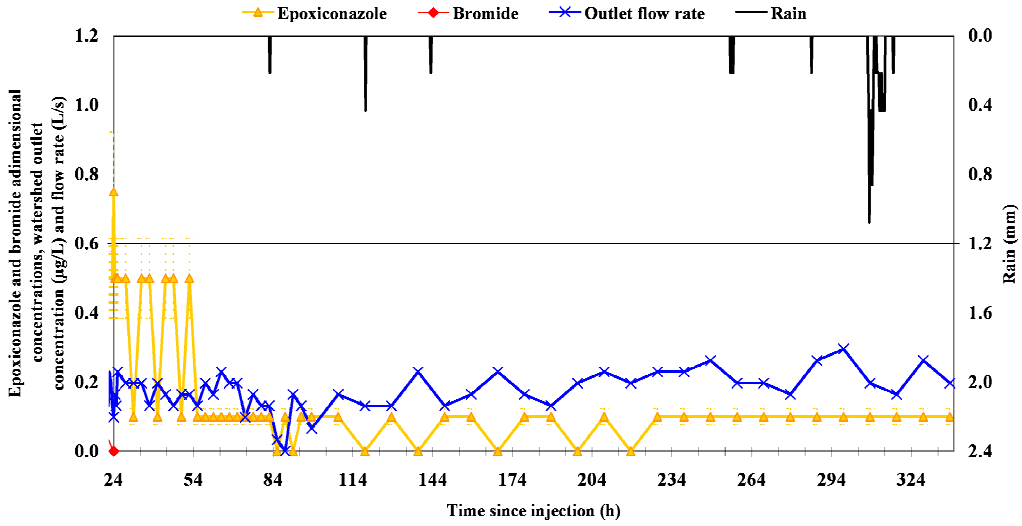

(a)



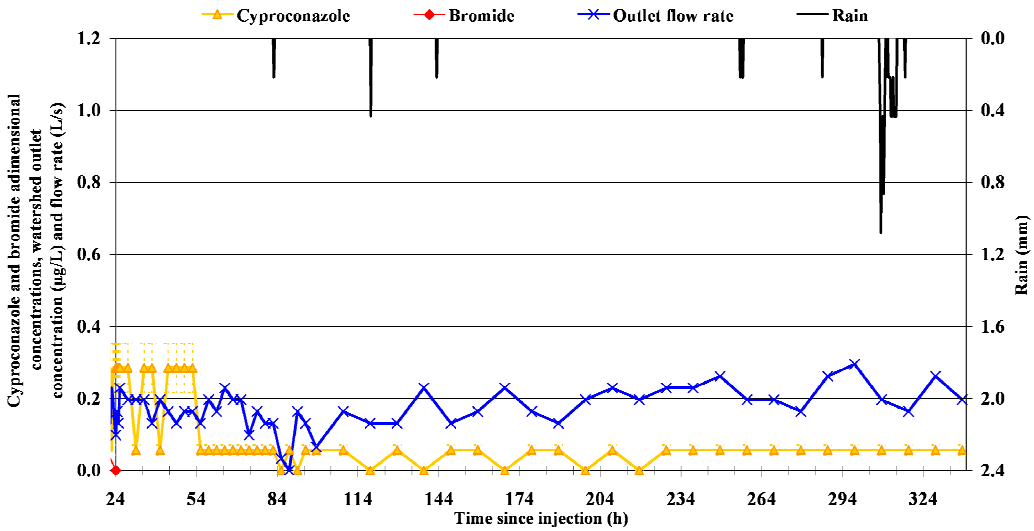

(b)


Metazachlor
Bromide
Outlet flow rate
Watershed outlet MTZ concentrations
Rain
Metazachlor and bromide adimensional concentrations, watershed outlet concentration (µg/L) and flow rate (L/s)
Rain (mm)
Time since injection (h)

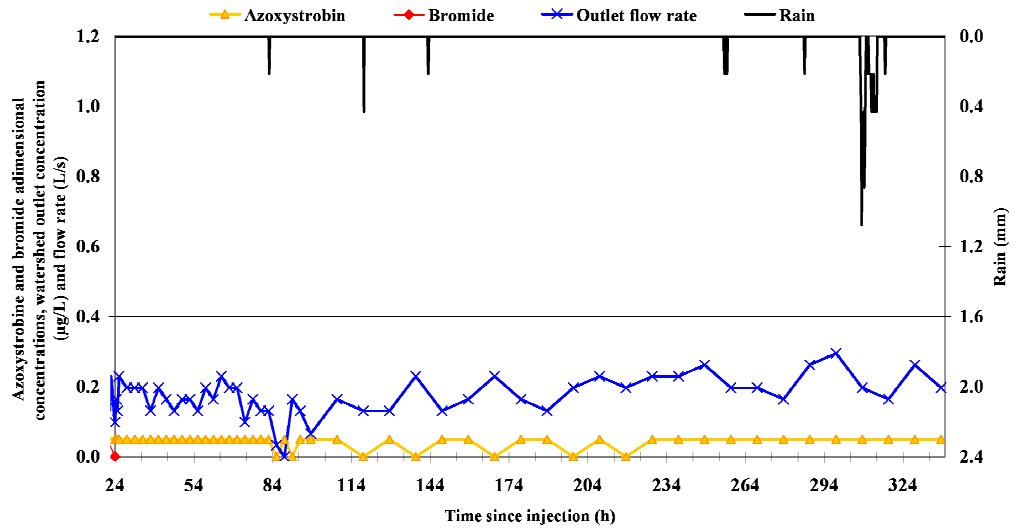
(c)


Epoxiconazole
Bromide
Outlet flow rate
Rain
Epoxiconazole and bromide adimensional concentrations, watershed outlet concentration (µg/L) and flow rate (L/s)
Rain (mm)
Time since injection (h)

(d)


Cyproconazole
Bromide
Outlet flow rate
Rain
Cyproconazole and bromide adimensional concentrations, watershed outlet concentration (µg/L) and flow rate (L/s)
Rain (mm)
Time since injection (h)

(e)




(f)

**Fig. III-20: Forest experimental plot outlet dynamics for pesticide parent molecules (yellow), metabolite (green), bromide (red) and flow rate (blue) for 24 to 340 h after injection. Rain is represented with a black thick line. Concentrations are normalized to maximal concentration values. ♦ are watershed outlet concentrations in grab water samples. (a) Isoproturon and desmethylisoproturon, (b) Glyphosate and AMPA, (c) Metazachlor, (d) Epoxiconazole, (e) Cyproconazole and (f) Azoxystrobin.**

### *4.3.1.b Discussion on a per-pesticide basis*

#### 4.3.1.b.i Glyphosate and AMPA

Contrary to glyphosate, AMPA was quantified as soon as the tracer experiment started. Despite glyphosate low half-life (on average 12 d), water temperature was low (6 °C on average), thus not being favourable for a fast degradation. It is therefore unlikely that measured AMPA came from injected glyphosate. In addition, AMPA was detected in grab water samples taken at the watershed outlet indicating that additional AMPA entered the experimental plot from the watershed. Glyphosate was used previously on the Bray watershed and may have entered the forest buffer in which it could have partially been degraded into AMPA. Glyphosate can adsorb on clay particles and aluminium or iron oxides and hydroxides, and is prone to fast degradation (Borggaard and Gimsing, 2008). Schnurer et al. (2006) showed that glyphosate de-carboxylation can take place even in its sorbed state. Transport of particles onto which AMPA and glyphosate were attached was demonstrated (Syversen and Bechmann, 2004). Syversen (2005) showed that a 5-m-grassed buffer had a 67 % AMPA retention due to solid particle sedimentation thus indicating that buffer zones could help glyphosate and AMPA dissipation.

#### 4.3.1.b.ii Isoproturon and desmethylisoproturon

Isoproturon recovery rate (41 % not-normalized, after 24 h and 72 % after 340 h) was very close to that found during the first tracer experiment (73 % after 135 h). However, for the same forest buffer, areal-normalized reduction *ATR* values were 0.15 %/m² (March 2008) and 1.09 %/m² (March 2009). Isoproturon concentrations and uncertainties were high leading to a fluctuating curve (Fig. III-19a). However, an overall decreasing trend seems apparent after isoproturon peak, up to approximately 7 h, before reaching a fairly steady state until 54 h after the start of the experiment. The steady state shows that isoproturon is slowly released from the forest experimental plot, and, most likely, continuously entering the system without strongly interacting with forest soil or litter. Isoproturon reversible adsorption was also confirmed in previous laboratory experiments (chapter II). In addition, the 4 March 2009 rainfall event was associated with isoproturon and desmethylisoproturon concentrations

increase presenting 3.8 ± 0.91 and 2.6 ± 0.62 µg/L peak concentrations, respectively. In addition, isoproturon was quantified at the limit of quantification (0.01 mg/kg) in forest soil and litter (Table III-7). Desorption was also suspected from the forest plot after March 2008 tracer experiment, as discussed in section 4.1.1. Laboratory experiments (Chapter II) showed that this molecule could be easily desorbed from such substrates after adsorption, as also noted elsewhere (Benoit et al., 2008). Margoum et al. (2001) studied isoproturon sorption on dead leaves and soil from an oak wood and found that increasing contact time enhanced isoproturon sorption, particularly on leaves. This "ageing" process may help a more strongly pesticide sorption with soil components over time (Gevao et al., 2000). Madrigal et al. (2007) showed that isoproturon biodegradation was larger in forest top soil horizon, presenting larger carbon and biomass content than deeper horizons, and thus more easily degradable. However, considering the two tracer experiments with high isoproturon recovery rates, degradation was probably low.

#### 4.3.1.b.iii Metazachlor

Despite presenting similar $K_{oc}$ values, metazachlor and isoproturon showed different behaviour. Metazachlor recovery rate was lower (30 %) than that of isoproturon, suggesting a greater sorption of the former than the latter. Laboratory experiments (Chapter II) concluded on similar sorption potential of forest soil and litter for the two herbicides. The difference between isoproturon and metazachlor behaviour in these results may be due to the continuous input of isoproturon load from the catchment. This may distort recovery rate calculations by over-estimating isoproturon outlet loads. However, metazachlor was also quantified at the catchment outlet with lower concentration values than those for isoproturon, thus also influencing load and recovery rate determinations. Metazachlor sorption was found to be weak but enhanced under the ageing effect (Mamy and Barriuso, 2007).

#### 4.3.1.b.iv Azoxystrobin

Azoxystrobin concentrations were low and rapidly decreased down to values lower than the LOQ. Load calculations showed high dissipation of this fungicide in the forest experimental plot. According to Ghosh and Singh (2009), azoxystrobin sorption increases with soil organic matter content. Bending et al. (2006) observed that even though azoxystrobin is non ionic, a decrease in pH can generate a decrease in sorption. This study also showed that azoxystrobin degradation is due to cometabolism processes which may have occurred thanks to the large availability of organic substrates in the forest buffer.

#### 4.3.1.b.v Cyproconazole

Azoxystrobin and cyproconazole had similar $K_{oc}$ values (Table III-2) but azoxystrobin seemed better dissipated than cyproconazole whose recovery rate was high (87 %, 24 h) in this tracer experiment. Some explanation could be found in their solubilities in water, that of cyproconazole (93 mg/L) being one order of magnitude higher than that for azoxystrobin (6.7 mg/L). This indicates that cyproconazole has a higher affinity than azoxystrobin for water. However, this is not corroborated by their $\log K_{ow}$ coefficients suggesting a reverse trend, confirming the complexity of pesticide molecules. A larger dissipation of cyproconazole in an organic matter-rich turfgrass soil than in bare soil was observed (Gardner et al., 2000), the former presenting the shortest half-life.

#### 4.3.1.b.vi Epoxiconazole

A fairly high dissipation of epoxiconazole was observed (33 % recovery rate, 24 h) which may be explained by its high $K_{oc}$ and $\log K_{ow}$ coefficients and low solubility (Table III-2). Epoxiconazole was detected on dead leaves at the forest plot inlet and middle zones 14 days after injection (Table III-7). This supports a possible adsorption of epoxiconazole onto

the forest soil or litter, as noted from laboratory experiments (Chapter II). However, despite low desorption was noted from laboratory experiments, additional 31 % of injected epoxiconazole was recovered between 24 and 340 h. However, load values present high uncertainties due to initial 23 % laboratory analytical uncertainties on epoxiconazole concentrations. Roy et al. (2000) showed that epoxiconazole hydrophobicity may explain its decrease in sorption for high moisture content soils which could account for epoxiconazole release or low adsorption.

#### *4.3.1.c Flow-weighted composite sample concentrations following the experiment*

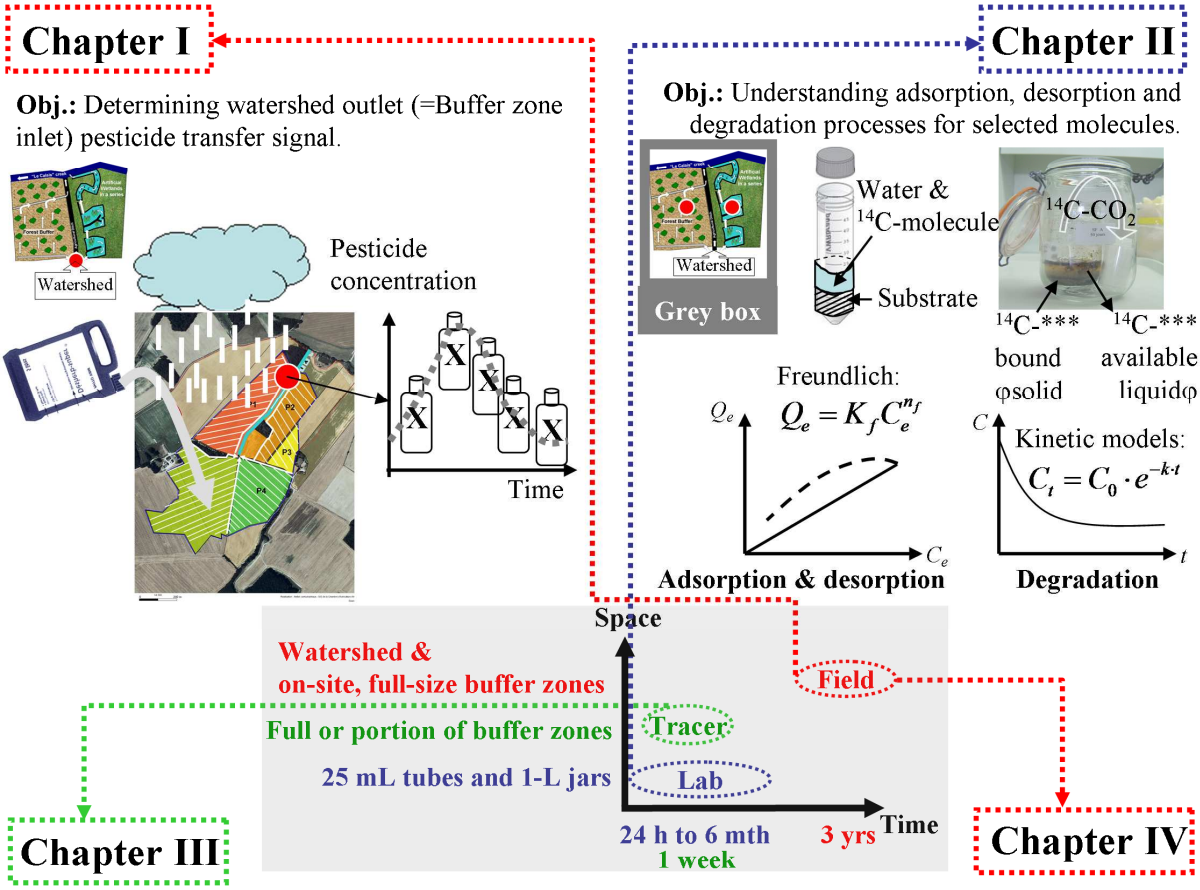
Isoproturon, metazachlor, cyproconazole and epoxiconazole belonged to the multi-residue SPME – GC/MS analytical method. In the first (10 March to 01 April 2009) and second (01 to 08 April 2009) flow-weighted composite samples taken after the tracer experiment, only isoproturon and epoxiconazole were detected. Concentrations in the first sample were 0.63 ± 0.05 (isoproturon) and 1.26 ± 0.10 µg/L (epoxiconazole); and 0.43 ± 0.03 (isoproturon) and 2.72 ± 0.17 µg/L (epoxiconazole) in the second. These values can not be only attributed to pesticide desorption from the tracer experiment as inlet concentrations were fairly high before the experiment was carried out (Chapter I).

## 5 Conclusions

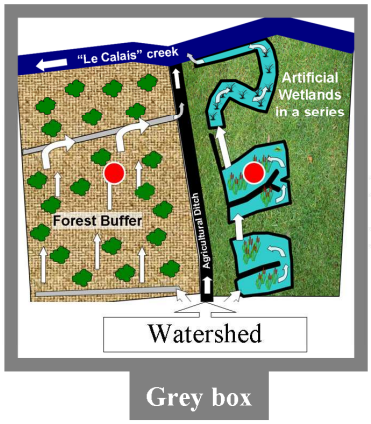
These tracer experiments confirmed that artificial wetlands and forest buffers can be attributed promising results for pesticide pollution mitigation. March 2008 multi-tracer experiment was carried out with a frequently measured low and constant flow rate. It showed good hydrological performance for artificial wetland with 66.5-h retention time and 87 % effective volume ratio whereas vegetation density was low. Hydraulic retention time is expected to decrease for large flow events but may have increased over time while vegetation density increased. Subsurface drainage blocking should also have prevented from further water and contaminant flows short-circuits. It appeared that uranine photodegradation occurred in the sparsely vegetated wetlands (81 % losses) as well as in the more shaded forest buffer experimental plot (57 % losses). The forest buffer tended to show high potential to reduce slightly sorbing molecules with 69 and 79 % of sulforhodamine B and isoproturon reduction, respectively. Such promising results for the forest buffer were confirmed by the March 2009 tracer experiment with load reduction varying from 36 (cyproconazole) to 78 % (metazachlor) after 24 hours. These results were not in agreement with pesticide sorbing properties suggesting that solubility, hydrophobicity and half-lives are among pesticide physico-chemical parameters that should be considered to understand their fate in the environment. In addition, as shown in chapter II, sorption may be a reversible process leading to pesticide desorption as suggested for isoproturon in the March 2008 experiment, and observed in the March 2009 study. Chapter II laboratory results were therefore good indicators of the nature of the processes that could be expected under more realistic dynamic conditions. Forest buffer seemed to present higher potential for pesticide pollution mitigation than the artificial wetland. However, as previously highlighted, forest buffers are likely to have lower water storage capacities. Consequently, they may not accommodate as many water volumes as the latter. Chapter I concluded that large water volumes are produced every year from artificially drained watersheds (> 60000 $m^3$ for Bray catchment) which make it impossible to treat them all in such buffer zones. The portion of water coming out of the watershed that could be caught and treated through buffer zones is another key component to assess whole treatment system efficiency, in addition to the internal system concentration or load reductions. Chapter IV focuses on this point as well as forest buffer and artificial wetland inlet versus outlet concentration and load reductions over the three years of monitoring.

# CHAPTER IV: BUFFER ZONES' FUNCTIONING WITH RESPECT TO PESTICIDE POLLUTION

## - - - - - - - - Dissertation outline diagram - - - - - - - -




**Obj.:** Understanding pesticide transfer in buffer zones under partially controlled dynamic conditions.




Mixing

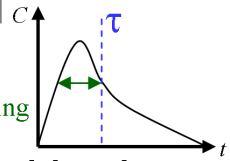
Short-circuits

Retention time

Pesticide transfer time

Pesticide retention %

Residence time



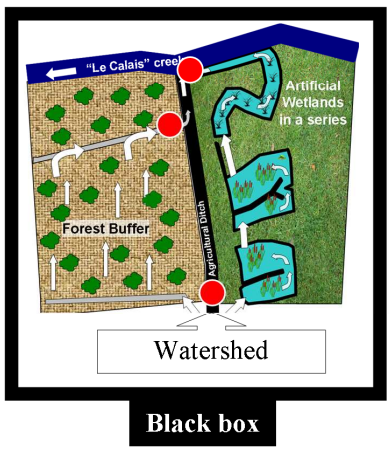

σ² = degree of mixing

**Tracer breakthrough curve**

● *You are here!*

**Obj.:** Determining on-site forest buffer and artificial wetland efficiency to reduce pesticide pollution.

**Real on-site conditions**




**Load** = Concentration × Volume

**Efficiency** = inlet vs. outlet (%)

## 1 Introduction

Assessing buffer zone performance implies determining overall pollution mitigation at large time (e.g., year) and space (e.g., macro-level watershed) scales under real climatic conditions. Most of previously published studies focused on inlet versus outlet buffer zone mass balances thus seeing them as "black boxes" (Appendix I). Among them and except for isoproturon, few studies were published on molecules of current interest like those studied in our laboratory (Chapter II) and tracer (Chapter III) experiments, most of research focusing on old pesticides, eg. atrazine, MCPA, diuron (Fig. IV-1). Introduction dates on the market for pesticides applied at Bray are significantly more recent ($\alpha$=0.05) than those for pesticides studied in the literature (29 references, Appendix I).

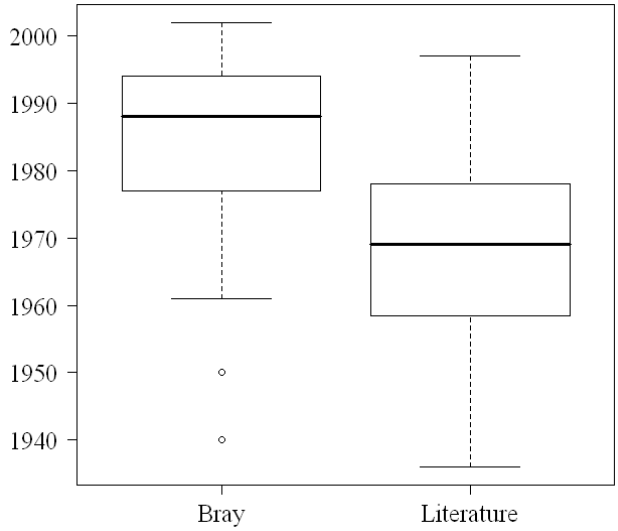



**Fig. IV-1: Introduction date distribution for pesticides applied on the Bray catchment ("Bray") or extracted from literature dealing with on-site artificial assessment for pesticide pollution removal ("Literature").**

Fig. IV-2 was made in an attempt to determine whether newly released pesticide molecules on the market exhibited different characteristics than "old" molecules. Only molecules cited in published papers and those applied at the Bray catchment (2002 – 2010) were selected ( n = 101) and data were extracted from the FOOTPRINT pesticide properties database (FOOTPRINT, 2010). A weak trend shows that young pesticide molecules are less soluble, more hydrophobic and more sorbing than older ones. Newly released molecules may therefore present a decreased leaching potential and an increased reduction in buffer zones through adsorption – desorption processes compared to older pesticides. However, these trends are definitely not significant as observed in Fig. IV-2.

Published literature did not discuss the reduced land availability issue cope with while implementing buffer zones in agricultural watersheds. Consequently, no strategy of selection of "flows of most concern" was assessed. Because this problem was faced at the Bray demonstration site, the proportion of total watershed pesticide loads that could in fact be caught and reduced within buffer zones was assessed. Positive and negative impacts due to the buffer zone presence were weighted studying modifications in receiving stream water quantity and quality. Moreover, it was attempted to link pesticide abatement results from in-situ long-term monitoring presented in this chapter to pesticide fate processes that can be expected from our laboratory (Chapter II) or tracer (Chapter III) experiments to start opening the "black box".

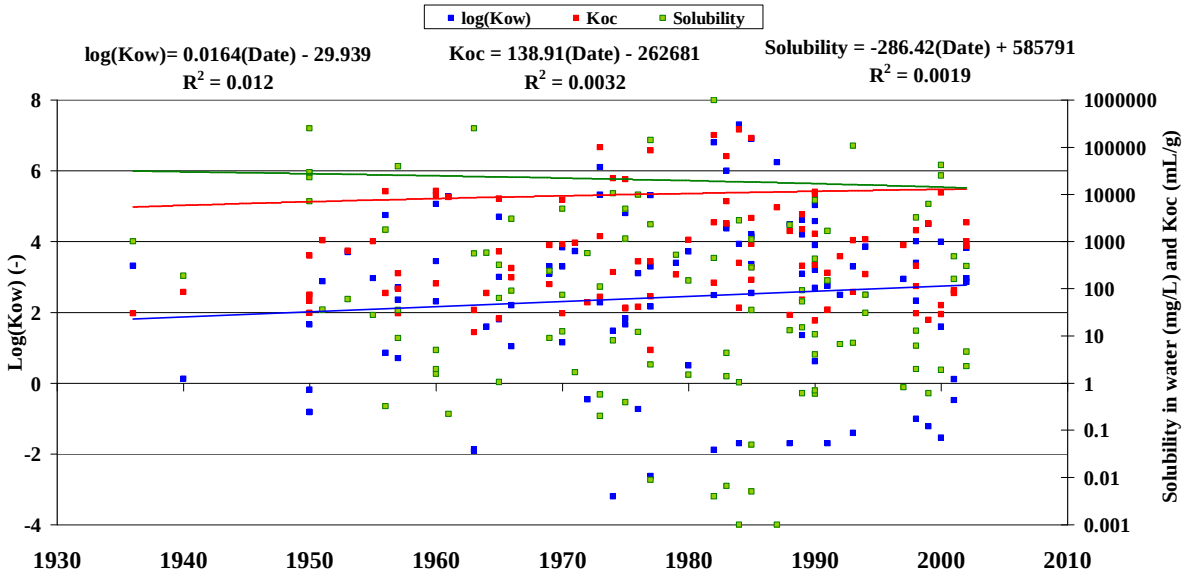



**Fig. IV-2: Evolution of pesticide hydrophobiticy (Log($K_{ow}$)), sorption properties ($K_{oc}$) and solubility in function of released date on the market.**

## 2 Methods

### *2.1 Foreword*

Determining buffer zone efficiency for water treatment may be challenging. Most studies used the "black box" perception comparing inlet and outlet concentrations or loads. Many of published results were carried out on a storm-by-storm basis and neglected to account for system volume capacity. Indeed, sampled outflow may present little or no relationship to the inflow of the same flow event leading to invalid comparisons between storm-by-storm calculated concentration of load reductions. Considering these observations, Strecker et al. (2001) recommended to statistically characterize inlet and outlet pollutant concentrations and loads without discrepancies between storm events. They also suggested that another appropriate evaluation would consist in taking as much water samples as possible to estimate total pollutant loads entering and exiting treatment systems. We attempted to take into account such recommendations for the Bray artificial wetland and forest buffer treatment efficiency evaluation.

### *2.2 Data analyzes*

A threshold of 1.5 L/s was selected to extract inlet peak flows for each individual flood. Probability plots and statistical analyzes were performed similarly as described for watershed outlet discharges (Chapter I). Nominal detention time ($T_n$, days) were calculated dividing wetland volume (330 $m^3$) by flow rate.

Flow-weighted composite samples taken at the inlets and outlets of the buffer zones were analyzed for sixteen molecules by SPME-GCMS (Passeport et al. (2010a), see Appendix IV). Loads of each pesticide j $L_j$ (mg) were calculated for each composite sample sampling period by multiplying pesticide j concentration (µg/L) by the volume that passed at the station (inlet or outlet) during the flow-weighted composite sample sampling period.

Concentration and load reductions ($\eta_{Cj}$ and $\eta_{Lj}$) for each pesticide j, evaluated from individual flow-weighted composite samples encompassing approximately one week, were calculated as follows:

$$\eta_{Cj} = \frac{C_{j\_in} - C_{j\_out}}{C_{j\_in}} \cdot 100 \qquad \text{(Eq. 27)}$$

Where $C_{j\_in}$ and $C_{j\_out}$ were pesticide j inlet and outlet flow-weighted composite sample concentrations, respectively. And,

$$\eta_{Lj} = \frac{L_{j\_in} - L_{j\_out}}{L_{j\_in}} \cdot 100 \qquad \text{(Eq. 28)}$$

where $L_{j\_in}$ and $L_{j\_out}$ were pesticide j inlet and outlet flow-weighted composite sample loads, respectively, estimated as presented in Chapter I. Concentrations below the limit of quantification (< LQ) were set to LQ/5, whereas those below the limit of detection (< LD) were set to zero.

Several precautions were taken while calculating concentration reductions. First, concentration reductions were not taken into account in buffer zones assessment when both inlet and outlet concentrations were below the limit of quantification (< LQ) (leading to 0 % reduction) or the inlet was < LQ and the outlet was below the limit of detection (< LD, leading to 100 % reduction). Indeed, for such low concentrations (< LQ or < LD), uncertainties are in the same order of magnitude resulting in the absence of significant difference between inlet and outlet concentrations. For instance, chlorotoluron LQ/5 is 0.02 ± 0.01 µg/L and zero (< LD) itself is associated with a 0.01-µg/L uncertainty. This implied that a zero or 100 % concentration reduction in such cases could be hardly claimed. In addition, taking into account these values would significantly affect the overall efficiency assessment through median concentration reduction calculations. Second, for pesticides used in tracer experiments, their concentrations in the first flow-weighted composite sample following the tracer experiment were not taken into account in mass calculations. It can be assumed that these concentrations are also due to pesticide injection from the tracer experiment which makes it difficult to link to the corresponding inlet flow-weighted composite sample. These concentrations were replaced by the average of the previous and next concentrations. A third criterion was imposed on water volumes. It was checked that during the sampling period of each composite sample (approximately every week), the water volume passing through the buffer zones was at least twice as large as the systems' volume to ensure that outlet and inlet composite samples corresponded to similar water volumes thus allowing their comparison.

Inlet and outlet concentrations and loads were usually not distributed normally as verified with Shapiro-and-Wilk tests ($\alpha = 0.05$). Histograms presenting watershed outlet concentrations (Fig. I-13) showed similar results. Possible significant differences between inlet and outlet concentrations or loads were detected by means of non-parametric Wilcoxon tests which did not require data normality using the R software (R Development Core Team, 2005).

Fig. IV-3 presents a diagram of inlet and outlet fluxes used to estimate overall 2007 – 2010 mass balances and pollutant reduction ($\eta_{Lj\_2007-2010}$):

$$L_{j\_WSout} = L_{j\_Ditch} + L_{j\_FBin} + L_{j\_AWin} \quad \text{(Eq. 29)}$$

$$L_{j\_FBin} = L_{j\_FBout} + L_{j\_FBremoved} \quad \text{(Eq. 30)}$$

$$L_{j\_AWin} = L_{j\_AWout} + L_{j\_AWremoved} \quad \text{(Eq. 31)}$$

$$L_{j\_Stream} = L_{j\_Ditch} + L_{j\_FBout} + L_{j\_AWout} \quad \text{(Eq. 32)}$$

$$\eta_{Lj\_2007-2010} = \frac{L_{j\_WSout} - L_{j\_Stream}}{L_{j\_WSout}} \cdot 100 \quad \text{(Eq. 33)}$$

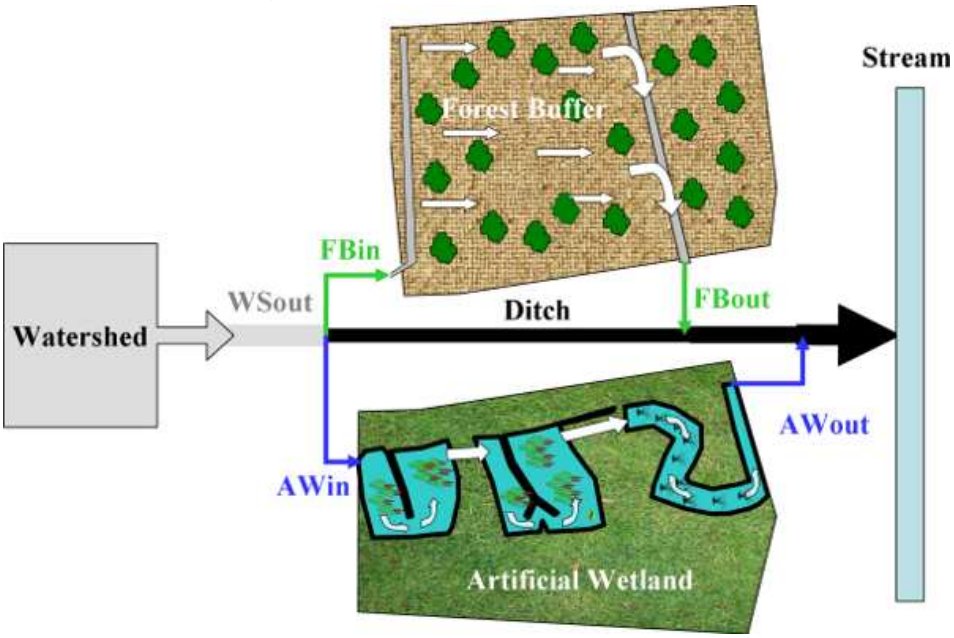



**Fig. IV-3: Diagram of flows and pesticides fluxes pathways. WS = Watershed, AW = artificial wetland, FB = forest buffer, in = inlet, out = outlet.**

Finally, toxic units (TU) were calculated for each molecule flow-weighted composite sample concentration as described in Chapter I.

## 3 Results and discussion

### *3.1 Buffer zone scale: internal efficiency*

As shown in chapter III, buffer zone efficiency is closely linked to internal hydraulics as it governs the travel time and pathway of water and pollutant molecules. Buffer zone global hydrology will be first described prior to presenting concentration and load reduction results.

#### 3.1.1 Hydrology

##### *3.1.1.a Wetland hydroperiod*

Wetland hydroperiod refers to temporal pattern of water level and saturation (Mitsch and Gosselink, 2000). Fig. IV-4 shows water level evolution in the three artificial wetlands in series (AW1 to AW3). The hydroperiod of the Bray wetland was dependent on rainfall distribution and wetland management through the open – close strategy. Such wetlands are characterized by the alternation of episodes of flooding and drying. Both aerobic and anoxic conditions may therefore prevail in the systems throughout the year.

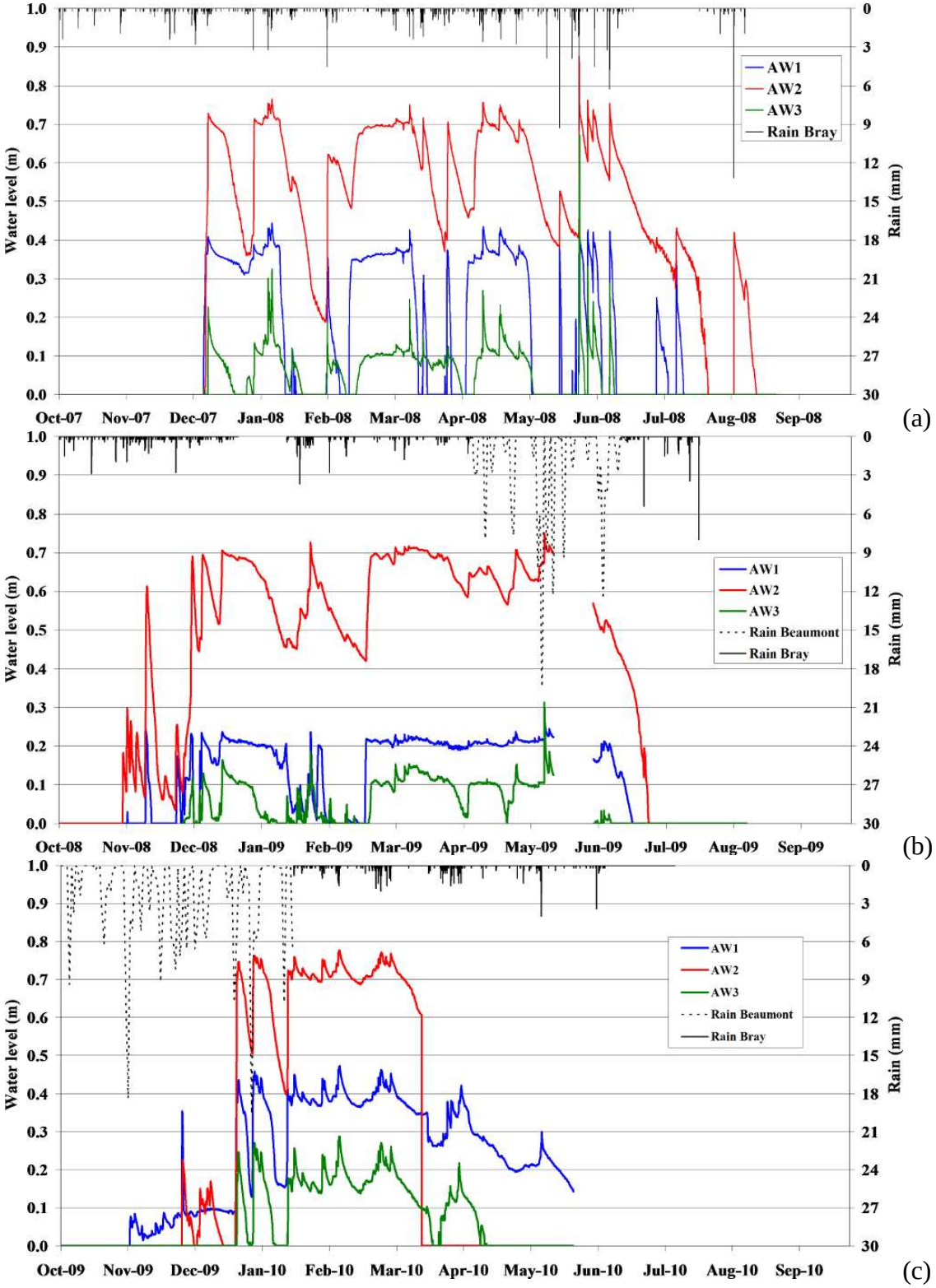



**Fig. IV-4: Water level fluctuation in the three artificial wetlands (AW) in series for (a) 2007 – 2008, (b) 2008 – 2009 and (c) 2009 – 2010 hydrologic years. "Rain" refers to rain measured on-site whereas "Rain Beaumont" corresponds to rainfall data from the closest Météo-France station.**

### *3.1.1.b Water volume reduction*

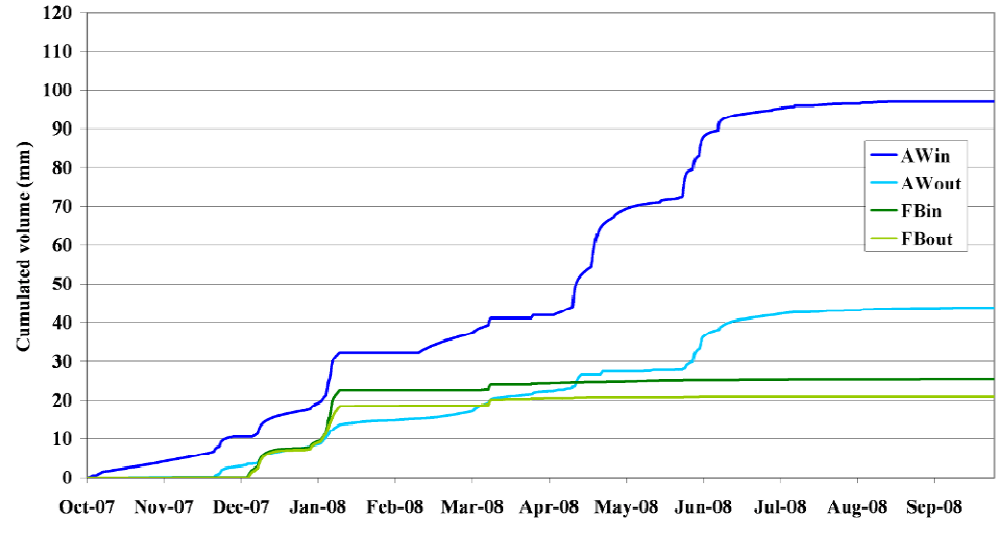
Yearly average water volume reductions ranged from 28 to 61 % in the artificial wetland and 25 to 28 % through the forest buffer. The lowest water volume reductions were recorded in the intense drainage seasons.

In artificial wetlands, water volume reductions accounted for 72 (2007 – 2008) and 100 % (2008 – 2009 and 2009 - 2010) during drainage initiation and 59 (2007 – 2008), 53 % (2008 – 2009) and 68 % (2009 – 2010) during the end of drainage period. In Fig. IV-5, it can be observed that differences between inlet and outlet volumes widen in spring. Water losses were therefore partly attributed to evapotranspiration. Vegetation creates shade, maintains humidity and reduces wind, however, such effects are offset by plant transpiration thus resulting in larger water losses from vegetated wetlands than from open-water systems (Kadlec and Wallace, 2008). Evapotranspiration rates vary along the year according to radiation and vegetation patterns. Roughly, a range of 2 to 10 mm/d was recorded according to vegetation type, period of the year, site location (Kadlec and Wallace, 2008). However, other leaks may have also occurred. Water losses during the intense drainage season were not null reaching 50 (2007 – 2008) and 48 % (2008 – 2009), whereas evapotranspiration was limited. Budd et al. (2009) also noted very high water volume reduction ranging from 68 to 87 % from a constructed wetland system in California (USA). Some infiltration probably took place under the artificial wetlands. Remaining underlying drains may also have accounted for such losses in spite of attempts to clog them in November and December 2008 (see chapter III). Lower losses were recorded during the 2009 – 2010 intense drainage season indicating that drain clogging may have been efficient in limiting these losses.

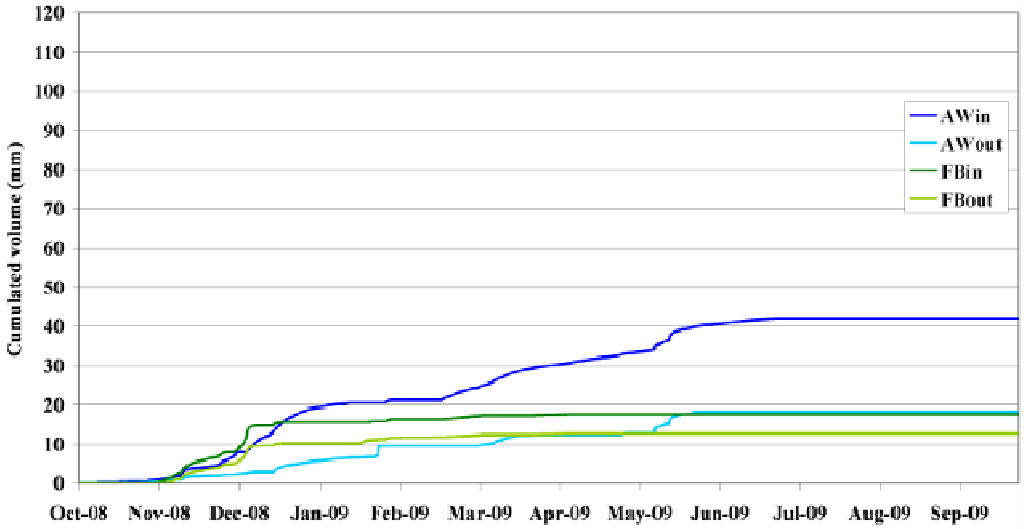
The forest buffer showed lower water volume reductions than the artificial wetlands. However, the forest buffer also received lower volumes than the wetlands. Moreover, contrary to the wetland system, it was not open in spring while evapotranspiration represents an important part of the water budget. During the intense drainage seasons, 25 (2007 – 2008) and 20 % (2008 – 2009) reduction were measured. These values were lower than that measured during the 2008 – 2009 drainage initiation period (38 %). During the other periods of the three years of monitoring, the forest was not opened for long time periods making difficult the comparisons. Leaks at the forest outlet were observed in late 2008. The lowest water losses in 2008 – 2009 may be the result of on-site intervention to adapt the same pipe diameter at the inlet than at the outlet to reduce the leaks.

### *3.1.1.c Peak flow rate attenuation*

In addition to water volume reduction, peak flow rate (> 1.5 L/s) reduction, one of hydraulic buffer system characteristics, is another important parameter to consider for describing hydrologic functioning, because it implies reduced erosion and sediment exportation to natural receiving waters (Kadlec and Wallace, 2008).




(a)

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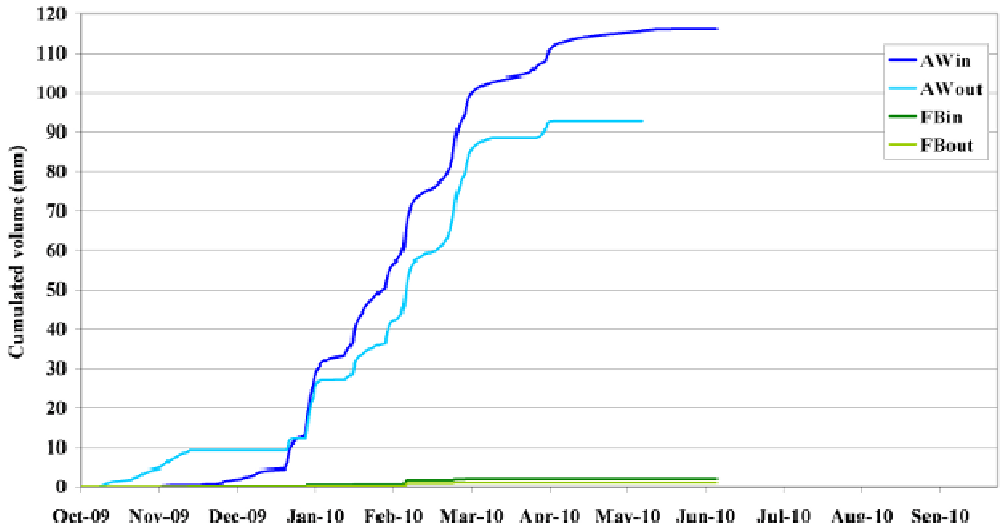


(c)

**Fig. IV-5: Cumulated volume at artificial wetland (AW) and forest buffer (FB) inlet (in) and outlet (out) for (a) 2007 – 2008, (b) 2008 – 2009 and (c) 2009 – 2010 hydrologic years.**

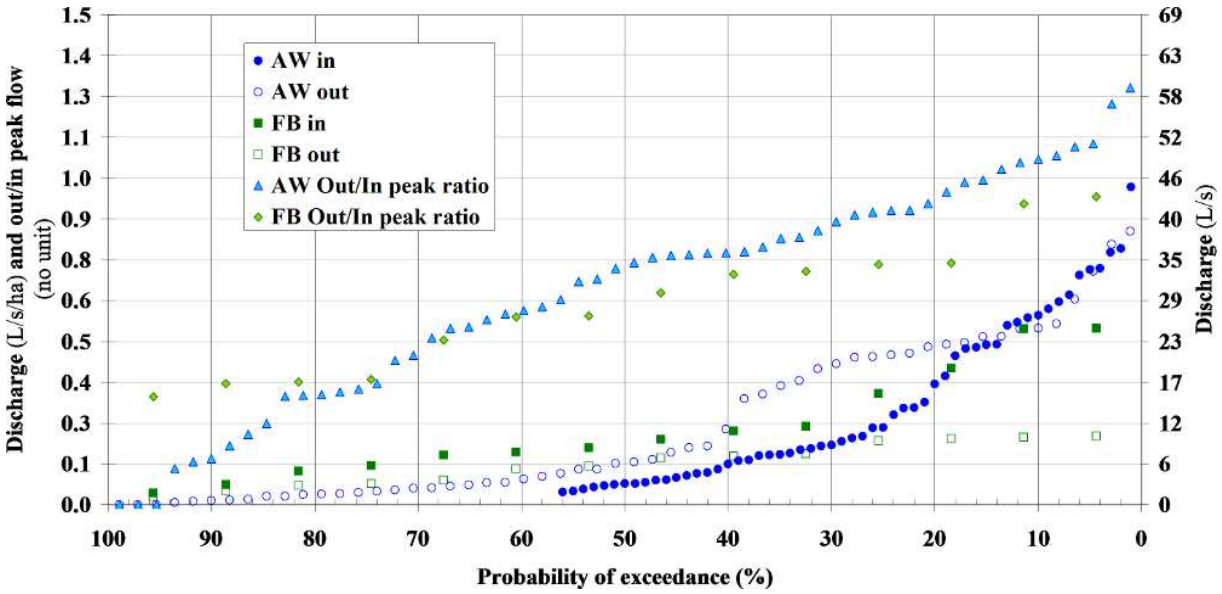


**Fig. IV-6: Probability of exceedance graphs for artificial wetland (AW) and forest buffer (FB) inlet (in) and outlet (out) discharges and outlet to inlet (Out/In) peak ratio.**

Fig. IV-6 presents a probability plot for peak flows recorded on both the artificial wetland and the forest buffer inlets and outlets for 2007 – 2010 hydrologic period. Peak flow values were gathered for the artificial wetland (n = 56) and the forest buffer (n = 14), respectively. All values are presented in Appendix VII. For the artificial wetland, median and mean peak discharges were 9.2 and 14.0 L/s (inflow) and 5.9 and 11.1 L/s (outflow), respectively. The forest buffer's median and mean values were 8.6 and 10.9 L/s (inlet) and 6.0 and 5.8 L/s (outlet), respectively. A 20-L/s peak flow rate was exceeded for 18 % of recorded floods, at each buffer zone inlet, whereas it was 29 % (AW) and 0 % (FB) at the outlets. Considering buffer zone design, inlet peak flow rates ranged from 1.8 to 44.8 L/s for the artificial wetland, whereas discharge peaks exiting the system ranged from 0 to 38.5 L/s. On the other hand, the forest buffer peaks ranged from 1.7 to 24.8 L/s at the inlet and 1.2 to 9.7 L/s at the outlet. Inlet flow rates rarely exceeded 35 L/s because of the inlet limited diameters. Artificial wetland outlet peak flow rate probability plots appeared to be above those of the inlet but it is important to note that such probability plot representations implies to first rank the data from the highest to the lowest value. It should therefore not be interpreted as systematic larger outlet than inlet peak flow rate. However, a limited number of rainfall events triggered overland flow resulting in extra water entering the artificial wetland through direct rainfall in the wetland and through runoff from surrounding cropped areas. It resulted in higher outlet than inlet peak flow rates on 14 % of the data. Outlet to inlet peak flow rate ratios, for individual storm events are also plotted on the same graph (Fig. IV-6). Most out/in peak ratio values are lower than one (left y-axis) demonstrating wetland and forest buffer efficiency at reducing peaks. In addition, at the inlet, forest flow peaks were lower than those of the artificial wetland because inlet pipes' elevation was slightly higher in the forest than in the artificial wetland. In addition, the forest buffer inlet pipe diameter was reduced on 05 March 2009 as described in chapter I thus decreasing inlet discharges. This could explain why the forest buffer appeared to mitigate peak flows better. Apart from extreme rainfall events, both systems proved to behave as hydraulic buffers. According to wetland soil initial condition before rainfall events, peak mitigation varied. For a previously empty artificial wetland (low water level), the fill-in effect tended to show better peak reduction than for a previously full system as shown in Fig. IV-7. It may therefore be recommended to help wetland system empty out to enhance their hydrological buffer qualities.

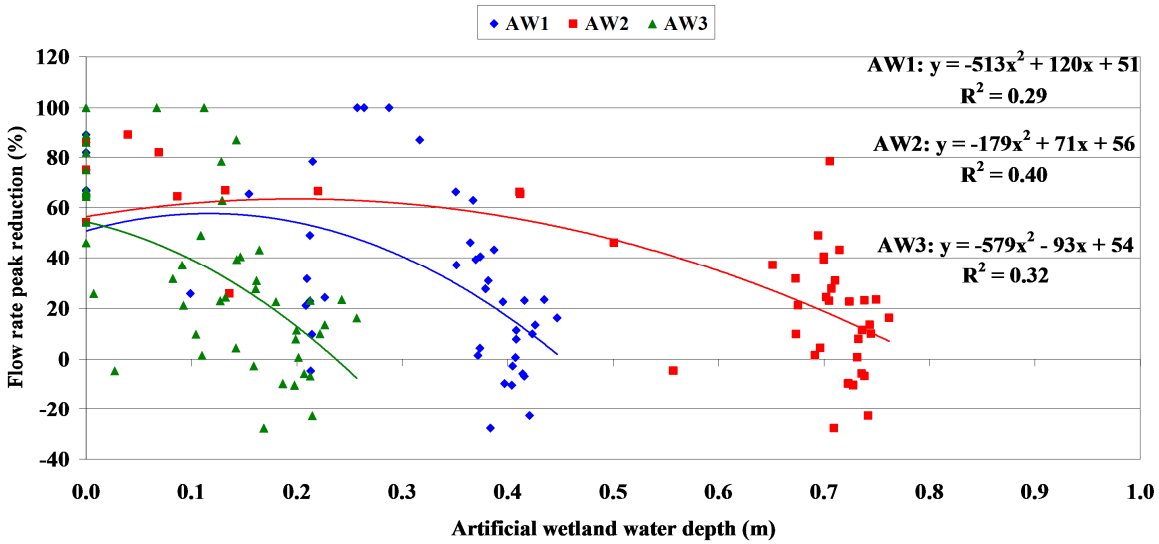


**Fig. IV-7: Flow rate peak reduction through artificial wetland (AW) according to water depth in each AW cell (AW1 to AW3).**

### *3.1.1.d Hydraulic retention time*

Water hydraulic retention times were variable due to extremely dynamic inlet flow characteristics of tile-drained catchments. The previously calculated 66.5-h residence time (chapter III) is only valid for similar flow conditions i.e. low flow rate of approximately 1.5 L/s. As wetland vegetation density increased since this tracer experiment was conducted, a longer residence time may be expected for such low flow conditions. However, during large floods, it is likely that water residence time decreased. Nominal detention times were calculated for the 56 recorded artificial wetland inlet peak flow rates (Appendix VII). Nominal detention times ranged from 2.0 to 51.5 h and the median was 10.0 h. It is important to note that these values correspond to peak discharges following rainfall events. Consequently, once the peak flow rate has passed, flow rate decreased leading to larger residence times. However, during most of the year, flow rates were steadier and occurred during flood tail or periods of no rain. Consequently, the 66.5-h residence time is likely to well represent periods of low flows but is definitely reduced during large storm events.

## 3.1.2 Pollutant abatement

### *3.1.2.a Pesticides*

Concentrations were usually lower than 5 µg/L (Fig. IV-8). Isoproturon, chlorotoluron, metazachlor and epoxiconazole exhibited the highest inlet concentrations. The other pesticide molecules were less frequently quantified and presented lower (usually < 2 µg/L) concentrations. As easily detected from box-plots and verified by means of Wilcoxon tests, inlet and outlet concentrations were not significantly different ($\alpha = 0.05$). The only significant difference was found for chlorotoluron inlet and outlet concentrations for the forest buffer. Very wide ranges of concentration reductions were found.

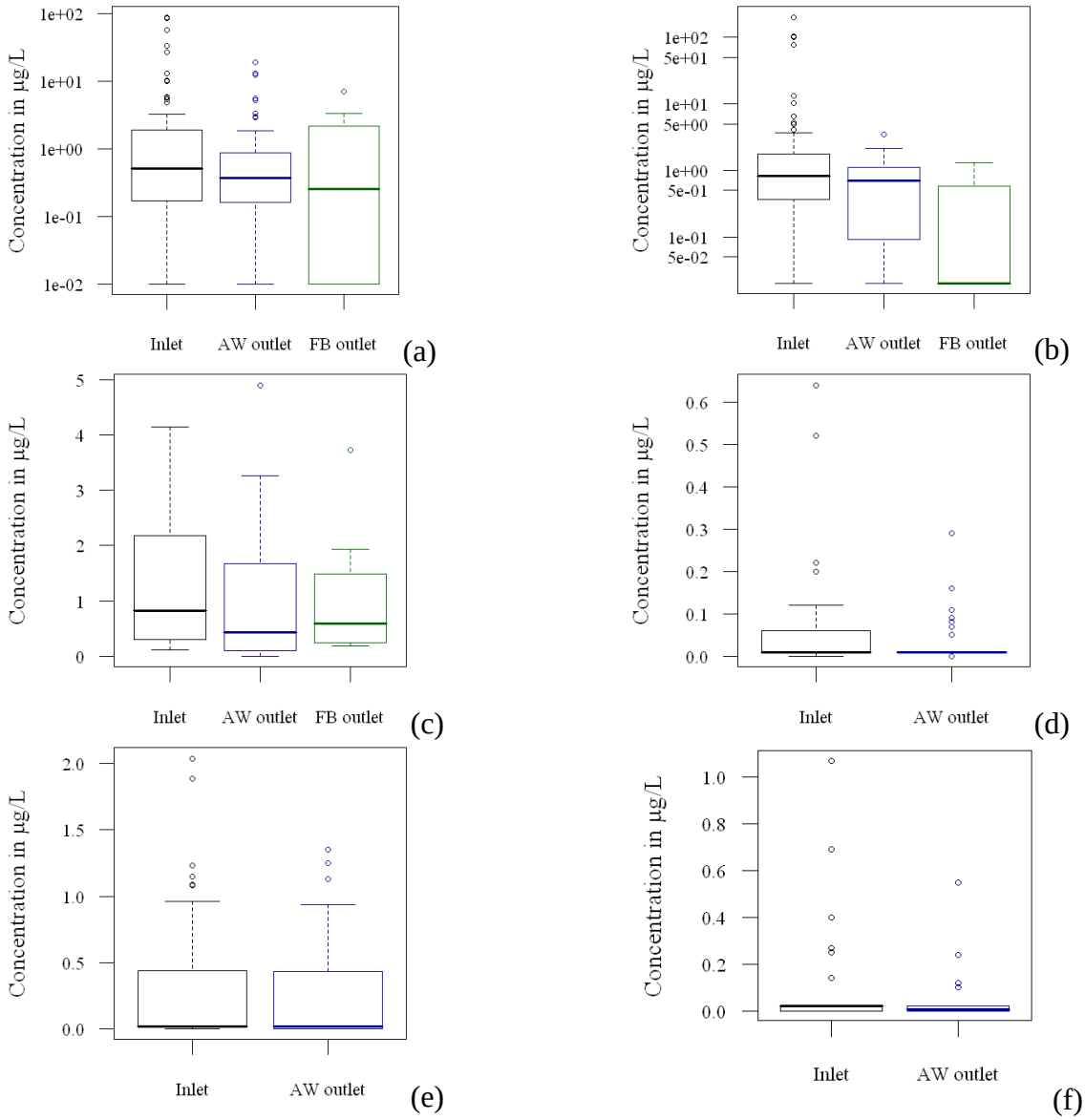


**Fig. IV-8: Inlet versus artificial wetland (AW) and / or forest buffer (FB) outlet concentration ranges for pesticides whose inlet-outlet pairs of samples were higher than 6. (a) Isoproturon, (b) chlorotoluron, (c) metazachlor, (d) diflufenican, (e) epoxiconazole and (f) tebuconazole. "Inlet" referred to the unique sampling station located in the agricultural ditch and corresponds to artificial wetland inlet, forest buffer inlet and watershed outlet. The bold black line is the median; the lower and upper boxes limits correspond to the first and third quartile which range is called the interquartile range. The whiskers' limits corresponded to 1.5 times the interquartile range. Outliers (circles) were set as data above the upper or below the lower whiskers.**

None of the flow-weighted composite samples exhibited high toxic units (larger than -1) either at the inlet or the outlet of the buffer zones. This indicates that the potential impact of the Bray catchment pollution to aquatic invertebrates such as *D. magna* was limited. However, it should be noted that flow-weighted composite concentrations were integrated over a period of approximately one week at Bray. Conversely, time-dependent samples may lead to concentrations that may reach larger values and therefore may be punctually harmful to receiving aquatic ecosystems. Monitoring pesticide ecotoxicological effects on-site is rarely carried out but should be recommended to better assess the real effects of measured concentrations.

| Molecule | 2007 - 2010 Concentration reductions | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Artificial Wetland | | | | | | Forest Buffer | | | | | |
| | Min | Max | Median | Mean | SD | n | Min | Max | Median | Mean | SD | n |
| Suspended sediments | -210 | 100 | 25 | -7 | 80 | 15 | -223 | 72 | 32 | -7 | 84 | 12 |
| Nitrate | -45 | 100 | 17 | 15 | 30 | 26 | -11 | 87 | 5 | 21 | 36 | 12 |
| Isoproturon[(a)] | -195 | 80 | -12 | -20 | 75 | 13 | -475 | 81 | 15 | -66 | 193 | 7 |
| Chlorotoluron[(a)] | -108 | 89 | 25 | 12 | 49 | 24 | 30 | 100 | 74 | 68 | 25 | 8 |
| Atrazine | 100 | 100 | 100 | 100 | | 1 | | | | | | 0 |
| Chlorothalonil | | | | | | 0 | | | | | | 0 |
| Prosulfocarb | 82 | 82 | 82 | 82 | | 1 | | | | | | 0 |
| Fenpropidin | 81 | 81 | 81 | 81 | | 1 | | | | | | 0 |
| Ethofumesate | 84 | 100 | 97 | 94 | 9 | 3 | | | | | | 0 |
| S-metolachlor[(a)] | 85 | 85 | 85 | 85 | | 1 | | | | | | 0 |
| Metazachlor[(a)] | -184 | 100 | 28 | 20 | 60 | 23 | -62 | 100 | 35 | 39 | 44 | 12 |
| Napropamide[(a)] | 6 | 12 | 9 | 9 | 4 | 2 | | | | | | 0 |
| Cyproconazole | | | | | | 0 | | | | | | 0 |
| Aclonifen[(a)] | 40 | 79 | 59 | 59 | 27 | 2 | 15 | 15 | 15 | 15 | | 1 |
| Diflufenican[(a)] | 14 | 88 | 47 | 50 | 30 | 6 | 18 | 98 | 20 | 45 | 46 | 3 |
| Tebuconazole | -106 | 100 | 46 | 38 | 74 | 7 | 67 | 86 | 76 | 76 | 13 | 2 |
| Mefenpyr-dietyl[(a)] | 42 | 42 | 42 | 42 | | 1 | | | | | | 0 |
| Epoxiconazole[(a)] | -191 | 56 | 25 | -2 | 75 | 9 | | | | | | 0 |
| Mean (pesticides) | | | | 47 | | | | | | 39 | | |
| Median (pesticides) | | | | 46 | | | | | | 28 | | |

**Table IV-1: Concentration reduction during the 2007 – 2010 monitoring period. [(a)]Molecules that had been applied at least once between 2007 and 2010. In bold are highlighted data for which the number of inlet – outlet pairs were higher than 8. Min, Max, SD and n stand for minimal values, maximal value, standard deviation (data dispersion) and number of inlet – outlet pairs.**

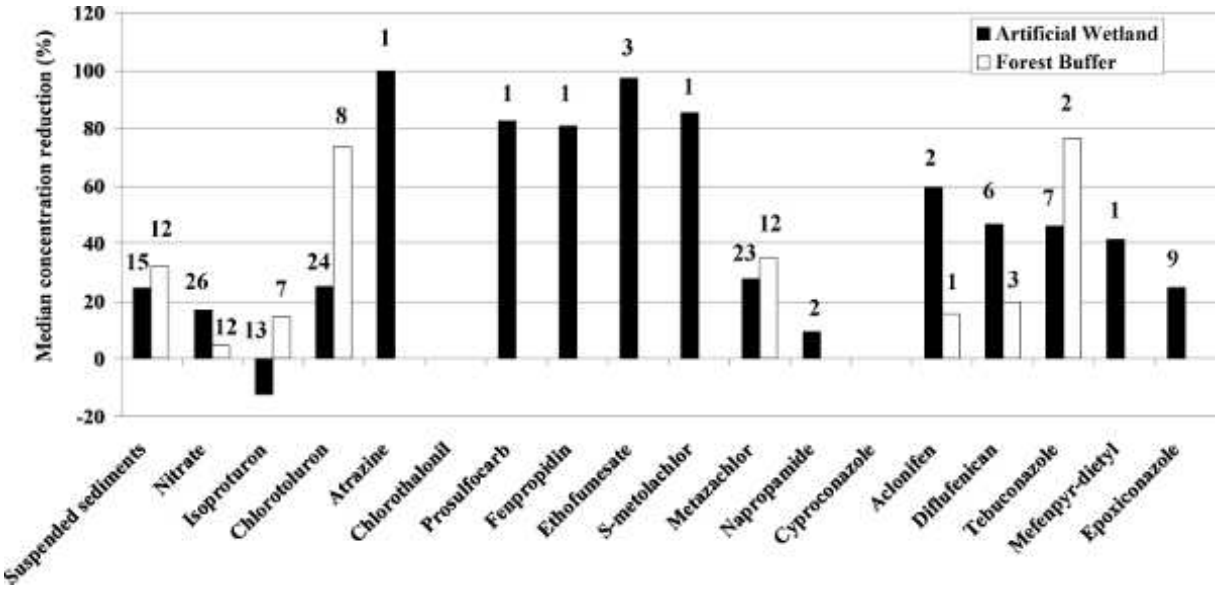


**Fig. IV-9: Median concentration reductions for suspended sediments, nitrate and the sixteen analyzed pesticides through the artificial wetland (black) and the forest buffer (white). Numbers above bars indicate the number of data (n) from which medians were derived. Chlorothalonil and cyproconazole did not present any pair on inlet – outlet concentration meeting the selected criteria.**

Concentrations reductions are presented in Table IV-1 and Fig. IV-9. During the 2007 – 2010 monitoring period, 42 and 12 inlet – outlet pairs of samples were collected for the artificial wetland and the forest buffer, respectively. However, all molecules were not detected or quantified in each sample. For instance, only 2 samples at the artificial wetland inlet exhibited concentrations higher than the limit of quantification for napropamide (Table IV-1). As detailed in Chapter I, despite being applied on the watershed in 2008 and 2009, this molecule was rarely quantified at the systems' inlets. Accordingly, when the number of data (n) was low, mean and median concentration reductions presented in Table IV-1 can not be considered reliable. On average, the artificial wetland and the forest buffer pesticide concentration reductions were 47 and 39 %, respectively. However, only considering pesticides for which more than six inlet – outlet pairs were collected, mean values were 16 (artificial wetland) and 14 % (forest buffer).

Altogether, concentration reduction median values for $n > 6$, ranged from -12 (isoproturon) to 47 % (diflufenican) in the artificial wetland and were associated with high standard deviations (Table IV-1). Previous authors also found such variable "efficiencies" (Miller et al., 2002; Haarstad and Braskerud, 2005; Hunt et al., 2008). Literature reviews usually report concentration-based reductions larger than 50 % on average for hydrophobic pesticides (Gregoire et al., 2008; O'Geen et al., 2010).

The forest buffer (for $n > 6$) had median concentration reductions of 15 (isoproturon), 74 (chlorotoluron) and 35 % (metazachlor). Despite a lower number of samples, it seemed that the forest better reduced pesticide concentrations as already suggested from laboratory results. However, no significant difference was found between the forest buffer and the artificial wetland outlet concentrations, except for the weakly sorbing herbicide chlorotoluron.

Pollutant load reductions (Table IV-2 and Appendix VIII) can be linked to Fig. IV-3 as detailed into Fig. IV-13 for an example (isoproturon) for a better understanding and Fig. IV-12. Higher load reductions were estimated compared to the previously discussed concentration reductions. On average, the artificial wetland and the forest buffer reduced pesticide loads by 73 and 54 %, respectively, over the 2007 – 2010 period. Load reductions were much larger than concentration reductions. This is partly due to concentration reductions through partly reversible adsorption or degradation. However, it may also be explained by large water losses observed through both buffer zones due to evapotranspiration and infiltration. Infiltration was shown to be the primary route driving pesticides' removal through grassed buffer strips (Lacas et al., 2005).

None of the three hydrologic period of each year appeared to clearly exhibit a larger potential than the other two ones for pesticide load reduction (Appendix VIII). A trend however seems to show that the highest load reductions were recorded during drainage initiation periods and the end of the drainage season. It is mostly explained by larger water volume reductions through these periods due to fill-in effect and increased evapotranspiration.

| Molecule | 2007 - 2010 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AWin | | AWout | | Annual reduction in AW | FBin | | FBout | | Annual reduction in FB | Ditch | WSout | Stream | Reduction[(d)] | |
| | load | AWin/ WSout (%) | load | AWout/ AWin (%) | % | load | FBin/ WSout (%) | load | FBout/ FBin (%) | % | load | load | load | load | %WS out |
| Water volumes[(a)] | 116749 | 50 | 63915 | 55 | 45 | 20742 | 9 | 14611 | 70 | 30 | 96956 | 234447 | 175482 | 58965 | 25 |
| Suspended sediments[(b)] | 17741 | 39 | 8040 | 45 | 55 | 2879 | 6 | 1326 | 46 | 54 | 24816 | 45436 | 34182 | 11254 | 25 |
| Nitrate[(b)] | 4319 | 46 | 2197 | 51 | 49 | 1155 | 12 | 750 | 65 | 35 | 3832 | 9306 | 6778 | 2528 | 27 |
| Isoproturon[(c)] | 210280 | 17 | 115529 | 55 | 45 | 59961 | 5 | 34072 | 57 | 43 | 947710 | 1217951 | 1097311 | 120640 | 10 |
| Chlorotoluron[(c)] | 431127 | 71 | 70688 | 16 | 84 | 29187 | 5 | 8212 | 28 | 72 | 148257 | 608572 | 227157 | 381414 | 63 |
| Atrazine[(c)] | 737 | 30 | 268 | 36 | 64 | 47 | 2 | 22 | 47 | 53 | 1636 | 2419 | 1926 | 493 | 20 |
| Chlorothalonil[(c)] | 4709 | 48 | 1007 | 21 | 79 | 560 | 6 | 264 | 47 | 53 | 4497 | 9766 | 5768 | 3997 | 41 |
| Prosulfocarb[(c)] | 4568 | 39 | 316 | 7 | 93 | 2066 | 18 | 0 | 0 | 100 | 5111 | 11745 | 5427 | 6318 | 54 |
| Fenpropidine[(c)] | 926 | 63 | 466 | 50 | 50 | 83 | 6 | 70 | 84 | 16 | 470 | 1479 | 1005 | 474 | 32 |
| Ethofumesate[(c)] | 4799 | 42 | 182 | 4 | 96 | 213 | 2 | 42 | 20 | 80 | 6432 | 11444 | 6656 | 4788 | 42 |
| S-metolachlor[(c)] | 477 | 60 | 96 | 20 | 80 | 77 | 10 | 18 | 23 | 77 | 245 | 799 | 359 | 440 | 55 |
| Metazachlor[(c)] | 110868 | 40 | 33131 | 30 | 70 | 28645 | 10 | 19087 | 67 | 33 | 137398 | 276910 | 189615 | 87295 | 32 |
| Napropamide[(c)] | 2508 | 66 | 874 | 35 | 65 | 356 | 9 | 119 | 33 | 67 | 936 | 3800 | 1929 | 1871 | 49 |
| Cyproconazole[(c)] | 5771 | 63 | 3218 | 56 | 44 | 218 | 2 | 288 | 132 | -32 | 3237 | 9226 | 6743 | 2483 | 27 |
| Aclonifen[(c)] | 2407 | 85 | 473 | 20 | 80 | 34 | 1 | 22 | 66 | 34 | 381 | 2821 | 876 | 1945 | 69 |
| Diflufenican[(c)] | 4801 | 68 | 1188 | 25 | 75 | 497 | 7 | 220 | 44 | 56 | 1777 | 7075 | 3185 | 3891 | 55 |
| Tebuconazole[(c)] | 6045 | 36 | 845 | 14 | 86 | 2249 | 14 | 674 | 30 | 70 | 8293 | 16588 | 9813 | 6775 | 41 |
| Mefenpyr-dietyl[(c)] | 3572 | 21 | 724 | 20 | 80 | 101 | 1 | 30 | 30 | 70 | 13402 | 17075 | 14156 | 2919 | 17 |
| Epoxiconazole[(c)] | 24075 | 28 | 6894 | 29 | 71 | 1833 | 2 | 436 | 24 | 76 | 60747 | 86655 | 68077 | 18578 | 21 |
| Mean (pesticides) | | 49 | | 27 | 73 | | 6 | | 46 | 54 | | | | | 39 |
| Median (pesticides) | | 45 | | 23 | 77 | | 5 | | 39 | 61 | | | | | 41 |

**Table IV-2: 2007 – 2010: Artificial wetland (AW) and forest buffer (FB) mass balances for the sixteen pesticides belonging to the analytical method. WSout, in and out stand for "watershed outlet", "inlet" and "outlet", respectively. [(a)] Water volumes are given in $m^3$. [(b)]Suspended sediments and nitrate units are kg. [(c)]Pesticide loads are in mg. [(d)]Reduction corresponded to the portion of pesticides that was actually dissipated through the two buffer zones and did not reach the stream.**

Among pesticides that were not applied during the monitoring period, concentration reductions could not be calculated for cyproconazole and chlorothalonil (n = 0). However, surprisingly, concentration reductions were calculated for atrazine, prosulfocarb, mefenpyr-diethyl, ethofumesate and fenpropidin (n < 3) and ranged from 42 to 100 %. This may be due to more ancient applications of such molecules. However, a possible explanation may arise from the analytical procedure. Indeed, it should be noted that these values mostly came from two samples whose sampling periods ended on 04 Dec. 2007 and 15 May 2009.[1]

Napropamide and aclonifen were applied on the Bray catchment and median concentration reductions (n = 2) were 9 and 59 %, respectively. These two pesticides have high $K_{oc}$ values but that of aclonifen (7126 mL/g) is much larger than that of napropamide (885 mL/g) which may contribute to the higher concentration reduction of the former.

For pesticides for which n > 6, sorbing and weakly soluble molecules (epoxiconazole, tebuconazole, diflufenican) appeared to show slightly larger concentration reductions than less hydrophobic molecules (isoproturon, chlorotoluron, metazachlor). Pesticide characteristics are therefore one of the driven factors that may explain wetland efficiency.

#### 3.1.2.a.i Weakly sorbing herbicides

On average (2007 – 2010), the lowest load reduction, similar to water volume reduction, was found for isoproturon. Concentration reduction mean value was negative in the forest buffer (- 66 %) and in the artificial wetland (- 20 %). It shows that, on some occasions, outlet concentrations were larger than inlet concentrations, particularly in the forest. Isoproturon is a moderately mobile herbicide that showed a very high frequency of quantification at both the watershed and buffer zones' outlets. Isoproturon has a moderate soil half-life ranging from 11 to 35 days in soils, but reaching 149 days in water/sediment studies (FOOTPRINT, 2010). The buffer zones' retention time was probably not large enough for isoproturon to be degraded. Tracer experiments showed that isoproturon reduction was higher in the forest (79 % in March 2008 and 59 % in March 2009) than in the artificial wetland (30 % in March 2008). However, forest outlet composite samples following tracer experiments exhibited high concentrations of isoproturon indicated it may have desorbed. Laboratory experiments supported this assumption. For such molecules, application rate reduction would be the key solution to reduce downstream pollution. This is in accordance with recent regulations regarding isoproturon use which is now forbidden on artificially drained catchments (AFSSA, 2007).

#### 3.1.2.a.ii Epoxiconazole: example of a sorbing fungicide

Despite precautions taken in inlet – outlet pair selection for concentration reduction calculations, wide variations were recorded from one composite sample to the next one thus preventing from highlighting any seasonal trend. This is illustrated with Fig. IV-10a and Fig. IV-10b detailing flow-weighted concentration evolution for epoxiconazole, applied on 15 April 2008. The next two composite samples had inlet concentrations of 0.96 ± 0.08 and 0.44 ± 0.04 µg/L while outlet concentrations were 0.43 ± 0.04 and 0.55 ± 0.05 µg/L, leading to +56 % and – 25 % concentration reductions, respectively. However, very similar concentrations were measured for the second sample considering associated analytical uncertainties. It clearly appears that April 22$^{nd}$ flood was sampled in between the two flow-weighted composite samples as the flow tail had not reached base flow before the end of the sampling period (Fig. IV-10a). An average concentration was calculated using the two flow-weighted composite sample concentrations and corresponding volume at the wetland inlet,

[1] Corresponding chromatograms from the GCMS analytical method were verified. An explanation may come from an accumulation of pesticides on the fiber layer material (PDMS/DVB) during the solid-phase micro-extraction procedure. However, blanks analyzed in the same series were of good quality.

and similarly at the outlet. Such calculations enabled covering the whole flood and led to an average concentration reduction of 38 %.

Similarly in June 2008, three pairs of inlet – outlet samples collected on June 3rd, 11th and 18th 2008, were associated with -191, +34 and -12 % concentration reductions. Again, the -12 % value was obtained from similar inlet (0.22 ± 0.03) and outlet (0.24 ± 0.03) concentrations. Alternation of positive and negative concentration reductions was frequently observed for this fungicide from one week to another. Despite high negative concentration reductions like -191 % value, all concentrations were lower than 1 µg/L at both the artificial wetland inlet and outlet. The extremely high flow rate of May 27th may have drastically decreased the wetland residence time and remobilized previously adsorbed epoxiconazole. Moreover, additional runoff and sediment-bound epoxiconazole entering the wetland from surrounding crop fields could also partly explain the -191 % value for this particularly large storm event. Considering an average concentration over June 11th and 18th composite samples, including the last two floods, an average concentration reduction of 27 % was calculated.

In this example, it also appears that the wetland outlet flow rate was sometimes affected by uncertainties due to deficiency in flow rate measurement by the electromagnetic flow meter. This may have also decreased data quality.

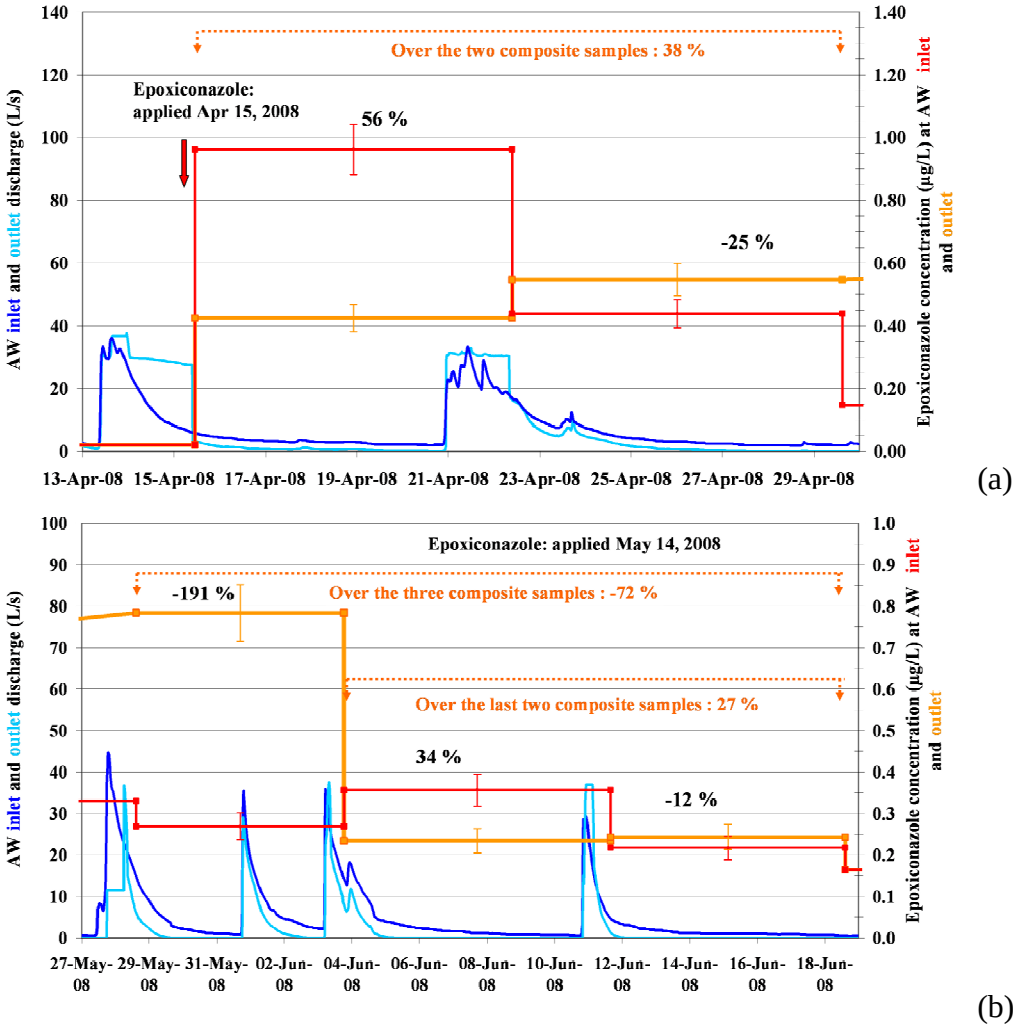


**Fig. IV-10: Epoxiconazole flow-weighted composite sample concentration at the artificial wetland (AW) inlet (red) and outlet (orange) for (a) April 2008 and (b) June 2008. Inlet (dark blue) and outlet (light blue) flow rates are also presented.**

The temporal evaluation scale should be adapted to assess buffer zone internal efficiency. When not centred on an individual flood, unreliable concentration reductions may be calculated. June 3rd composite sample was made of an individual flood (June 1st), the tail of a previous large flood (May 27th) and the peak of June 3rd flood. May 27th flood inlet peak

was not part of the inlet composite sample whereas outlet peak was sampled in the corresponding outlet composite sample. This sample was therefore a complex mixture of water and pesticides. No correlation between nominal detention times and efficiencies could be extracted from Table IV-3.

| Sampling date | Inlet | | Outlet | | $\eta_C$ |
|---|---|---|---|---|---|
| | Q in (L/s) | $T_n$ in (h) | Q out (L/s) | $T_n$ out (h) | % |
| 22 Apr 08 | 7.28 | 13 | 6.89 | 13 | 56 |
| 29 Apr 08 | 4.72 | 19 | 2.5 | 37 | -25 |
| 3 Jun 08 | 7.21 | 13 | 3.88 | 24 | -191 |
| 11 Jun 08 | 4.21 | 22 | 2.41 | 38 | 34 |
| 18 Jun 08 | 1.37 | 67 | 0.03 | 3056 | -12 |

**Table IV-3: Average flow rate (Q) at the wetland inlet (in) and outlet (out), calculated nominal detention times ($T_n$) and concentration reduction ($\eta_C$).**

In the artificial wetland, on average, 71 % of epoxiconazole loads were dissipated whereas it was 76 % in the forest buffer. From the results obtained in the laboratory experiments, it is likely that epoxiconazole load reduction be mainly due to its partially irreversible sorption. However, despite not being negligible, only 25 % (median) of epoxiconazole concentrations were reduced in the artificial wetland. These results do not reinforce those obtained at the laboratory scale confirming that up-scaling is extremely difficult. Indeed, laboratory experiments concluded on large retention potential of buffer zones for hydrophobic pesticides like epoxiconazole due to weakly reversible adsorption. However, on-site March 2009 tracer experiments led to 67 % epoxiconazole reduction in the first 24 hours and 33 % release in the following two weeks (chapter III), which was larger than what was expected from laboratory results (chapter II). Finally, the present on-site, large time and space scale study shows that compared to the other molecules, epoxiconazole does not exhibit a much larger potential at being reduced. Considering smaller scale results from chapters II and III, it can be thought that epoxiconazole and substrates contact was not large enough to result in significant reduction of the fungicide. Runes et al. (2003) studied atrazine removal through constructed wetlands and also highlighted that laboratory results on atrazine adsorption overestimated those measured on-site.

#### 3.1.2.a.iii Relationship between pesticide mitigation and hydrology

Negative concentration reductions indicated the occurrence of larger concentrations at the outlet than at the inlet of the buffer zone. As observed from laboratory experiments (Chapter II), a major process governing pesticide fate, particularly for large floods during which low residence times occurred, is pesticide adsorption and possible further desorption. Accordingly, it is likely that some of the pesticide molecules first adsorbed onto artificial wetland and forest buffer substrates prior to desorbing. In addition, as shown in Chapter III, dead zones were observed in the artificial wetland. A portion of pesticide loads could also be temporarily stored in such zones of slow exchanges before being remobilized later on. This could generate higher outlet concentrations than those at the inlet for the same sample collection date. As presented in the method part of this chapter, samples taken after tracer experiments were not used for concentration reduction calculations. Concentrations were also affected by uncertainties which appear on graphs presented in Appendix IX.

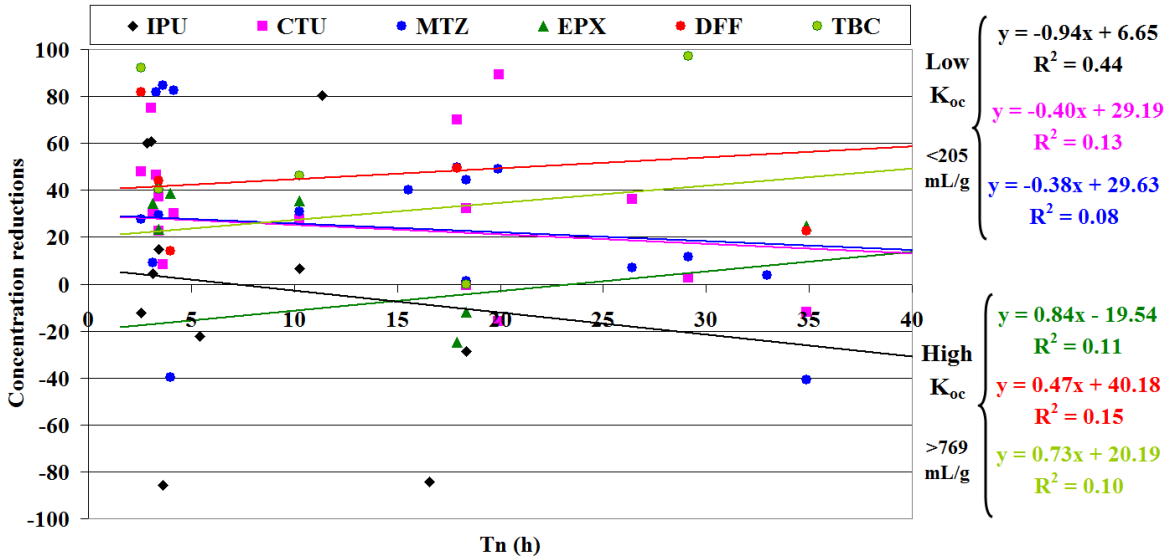


**Fig. IV-11: Concentration reduction in the artificial wetland according to nominal detention times ($T_n$) based on artificial inlet peak flow rate for isoproturon (IPU), chlorotoluron (CTU), metazachlor (MTZ), epoxiconazole (EPX), diflufenican (DFF) and tebuconazole (TBC).**

From Fig. IV-11, it seems that concentration reduction for moderately sorbing molecules such as isoproturon, chlorotoluron and metazachlor decreases with nominal retention times, whereas the reverse trend was found for sorbing molecules like epoxiconazole, diflufenican and tebuconazole. Nevertheless, these results were based on a narrow range of nominal retention times themselves calculated from inlet peak flow rates and not individual and repeated tracer experiments under different flow rate conditions. Very low correlation coefficients were found. However, the objective was to attempt to extract some tendencies and understand pesticide fate in the artificial wetlands, given the previous results from laboratory or tracer experiments.

The surprising negative relationship for the least sorbing pesticides may be explained by the fact that even a 40-h nominal residence time is not long enough for these molecules to strongly adsorb or degrade. However, as efficiency seems to decrease, it may be due to desorption that had time to occur while increasing residence time. Finally, it is important to note that the three herbicides were those that presented among the highest quantification frequencies at the watershed outlet. They continuously entered the system which may affect concentration reduction values despite precautions taken on water volumes (330 $m^3 \times 2$) for calculations.

Adsorption is supposed to occur at a faster rate than degradation for most molecules. The positive correlation found for hydrophobic molecules may indicate that the artificial wetland is likely to be efficient for hydrophobic pesticides sorption. This is in agreement with laboratory experiments showing that from wetland sediments or plants, a less reversible sorption for epoxiconazole than for isoproturon or metazachlor was expected (chapter II).

In addition, epoxiconazole was shown to form non-extractable residues through time which may contribute to its reduction and can also be expected from similarly hydrophobic compounds. Only once in March 2009, sediments and forest soil were sampled to determine pesticide concentrations in this compartment. All results were lower than 0.01 mg/kg (limit of quantification) for epoxiconazole, metazachlor, azoxystrobine, cyproconazole, isoproturon and two of its metabolites, and glyphosate and its AMPA metabolite. Such analysis should be repeated to determine whether pesticides are accumulating or not in wetland sediments through time until being degraded or released to water column. A study conducted in a storm water wetland in another of the ArtWET project demonstration sites concluded that some pesticides accumulated in sediments like tetraconazole (Maillard et al., Submitted). However, after concentration increase in sediments, others appeared to be further degraded.

These results may argue for the artificial wetland being under-sized with respect to pesticide pollution abatement. The same observation cannot be made for the forest buffer whose efficiency, based on a much lower number of samples however, showed promising results.

It should be highlighted that these values were all derived from flow-weighted composite samples collected approximately every week. Despite the condition set on water volumes (> 330 $m^3$ × 2), to allow for pesticide concentration reduction calculations, discussing concentration reductions is difficult. Indeed, as discussed in chapter I, wetlands located at tile-drained catchment outlets present a specific hydrology characterized by large but variable inflows in winter and dry periods in summer. Intra-annual variability in inflows and pesticide inlet loads is dependent on application timing and rainfall events. Each flow-weighted composite sample may not isolate a specific storm event for which inlet and outlet sampled water volumes would be easily comparable.

#### *3.1.2.b Suspended sediments and nitrate*

Nitrate and suspended sediment concentrations were not the first criteria that led the Bray buffer zones' implementation. However, their evolutions were monitored to detect possible additional effect of the systems. Nitrate concentration reduction mean and median values were 15 and 17 %, respectively in the artificial wetland. Results were 21 (mean) and 5 % (median) reduction for the forest buffer. This is lower than what is usually found in the literature (approximately 40 %) (Hammer and Knight, 1994). Both positive and negative concentration reductions were observed as previously found elsewhere (Nahlik and Mitsch, 2006). Similarly, suspended sediment concentrations were also slightly dampened within the systems with 25 (artificial wetland) and 32 % (forest buffer) median concentration reductions. This may however have helped a low portion of sediments-bound pesticides like glyphosate to be reduced through particle sedimentation (Syversen and Bechmann, 2004).

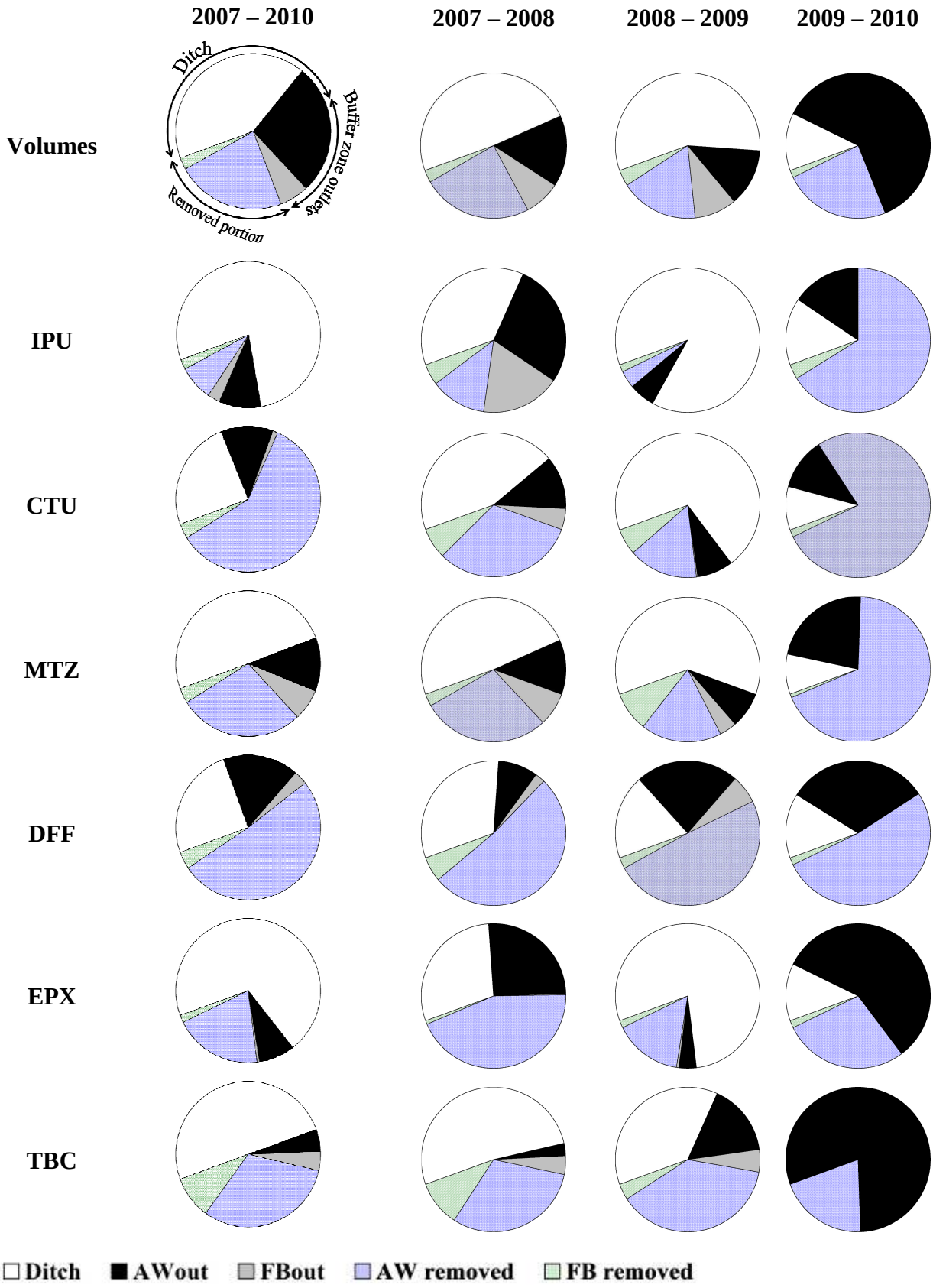


**Fig. IV-12 : Distribution of watershed outlet (total cicles) water volumes (volumes) or loads of isoproturon (IPU), chlorotoluron (CTU), metazachlor (MTZ), diflufenican (DFF), epoxiconazole (EPX) ad tebuconazole (TBC) into the agricultural ditch (Ditch, white), the portions removed in the artificial wetland (AW removed, blue grid) and the forest buffer (FB removed, green hatchings) and the part crossing the systems and being measured at the artificial weltand outlet (AWout, black) and the forest buffer outlet (FBout, grey) for 2007 – 2008, 2008 – 2009 and 2009 – 2010 hydrologic years.**

## *3.2 Watershed scale: global efficiency*

### 3.2.1 Open – Close strategy

The buffer zones were located parallel to the main ditch. They could only receive water from the catchment when the pipe elbows located in the main ditch were down-turned by the farmer after its pesticide applications. Fig. IV-12 shows how watershed outlet water volumes and pesticide loads partitioned between the agricultural ditch and the buffer zones. The parts removed by or exiting the buffer zones are specified.

#### *3.2.1.a Hydrology*

The portions of watershed outlet water volumes that passed through the artificial wetland and the forest buffer were on average 50 and 9 %, respectively, during the whole 2007 – 2010 period (Table IV-2). Similar portions of water volumes passed through the systems during the first two years of monitoring, resulting in 41 and 31 % for the artificial wetland and 11 and 13 % for the forest buffer, for 2007 – 2008 and 2008 – 2009, respectively (Appendix VIII). Along year 2009 – 2010, the artificial wetland was particularly efficient at catching water volumes (86 % of watershed outlet) whereas the forest buffer was almost not open (Fig. IV-12). These values are the result of our scheduled management consisting in preventing water from entering the forest buffer in spring, in order to preserve tree growth. A better knowledge of the effect of repeated flooding on the forest buffer vegetation growth may have helped optimizing the open – close strategy for the forest. The artificial wetland was closed approximately one month after fall pesticide application to give time to the system to drain and be empty to store and treat flows following spring pesticide applications. Consequently, the artificial wetland was open and caught most water volumes during drainage initiation period, and some volumes during the intense drainage season.

#### *3.2.1.b Pesticides*

On average over the three years of monitoring, the artificial wetland and the forest buffer collected 50 and 6 % of pesticide loads measured at the watershed outlet, respectively (Table IV-2). In 2007 – 2008, pesticide loads entering the artificial wetlands during drainage initiation period were similar to those caught during the intense drainage season (Appendix VIII). Conversely, the intense drainage season was associated with the largest loads in the next two years. These trends reflect the volumes of water generated at the catchment outlet during these two hydrologic periods. For instance, 2007 – 2008 drainage initiation was associated with larger volumes (10840 $m^3$) than during 2008 – 2009 (6252 $m^3$) and 2009 – 2010 (2080 $m^3$) periods.

Isoproturon and chlorotoluron were the two pesticides exhibiting the highest loads entering the buffer zones with 11 to 340 g at the artificial wetland inlet and 1 to 42 g in the forest buffer. Metazachlor loads at the systems' inlets varied from 46 mg to 97 g and those of epoxiconazole ranged from 19 mg to 13 g (Appendix VIII). This is in agreement with the application rates of these molecules (Appendix VIII).

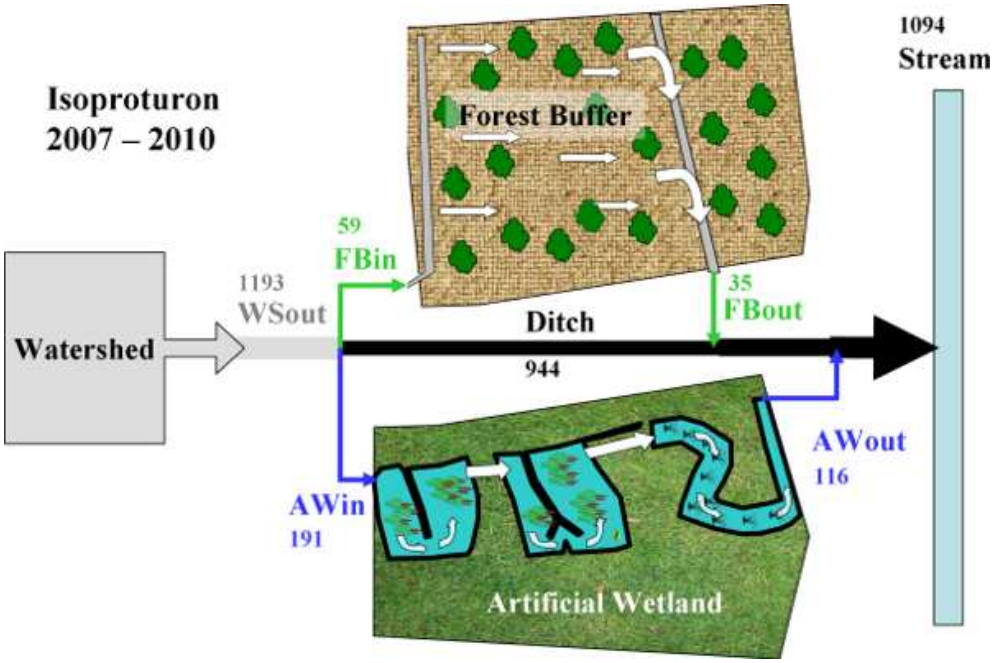


**Fig. IV-13: Isoproturon mass balance (in mg) diagram for the 2007 – 2010 period of study.**

Overall, it can be observed that most isoproturon loads entered the artificial wetlands during drainage initiation or end of drainage seasons. However, this was variable according to pesticide application and rainfall timings. For instance, in 2007 – 2008, approximately 40 % of watershed outlet isoproturon loads entered the artificial wetland during the three hydrologic period of the year. In 2008 – 2009, the buffer zones were open after isoproturon application in December 2008 that occurred later than usual (10, 22 and 23 December 2008). A period of frost delayed watershed outlet flows thus significantly reducing them in winter 2008 – 2009. The buffer zones had been closed mid-January whereas the flows presenting the highest loads of isoproturon had not occurred yet (Fig. I-12 and Appendix VIII). Consequently, most isoproturon loads entered the wetlands once opened again during the intense drainage season when concentrations were high, as discussed in Chapter I. Indeed, only 26 mg entered the wetland in 2008 – 2009 drainage initiation period whereas 92048 mg passed during the intense drainage season (Appendix VIII).

There is an important uncertainty on isoproturon (and chlorotoluron) concentrations in the flow-weighted composite samples following this application. Indeed, after a first analysis, concentrations appeared to overpass the highest value of the validated analytical range (0.05 – 5 µg/L) for this molecule. Samples were therefore diluted and analyzed again but concentrations looked lower than expected. As noted while developing the analytical method (Passeport et al., 2010a), the method specificity is poor, particularly for isoproturon and chlorotoluron. This indicates that sample matrix (water from agricultural areas) may include substances that may increase isoproturon and chlorotoluron response during the analysis. Dilution may have dampened this effect so that a different response was obtained.

For chlorotoluron, large and fairly equal loads entered the wetlands during the 2009 – 2010 drainage initiation and intense drainage seasons leading to 213 and 123 g, respectively. Between 19 and 35 g of chlorotoluron load evenly entered the artificial wetland along 2007 – 2008 hydrologic year whereas the previous application was in November 2005.

Epoxiconazole is a spring fungicide applied every year at the Bray catchment. It was noted in Chapter I that after a period of low flows, while drainage started again, high concentrations were measured. This explains why, even during intense drainage season, high epoxiconazole loads were caught (39 mg to 5.8 g) after the wetland stayed close for a long time. The highest epoxiconazole loads entering the artificial wetlands were however recorded during the "end of drainage" period, following its application. The low 2009 – 2010 value was due to the fact that only one sample was collected at the catchment outlet after epoxiconazole

application (08 May 2010) because the drainage season ended earlier than usual. Targeting the flows following pesticide applications seemed to perform well for the artificial wetland. The low contribution of the forest buffer to pesticide abatement was mainly due to the fact that it had been voluntarily closed in spring to preserve tree growth. Consequently, it could mostly receive the herbicides that were applied in fall or winter from flows occurring during drainage initiation or intense drainage seasons.

However, as stated previously, some pesticides were quantified even long after their application, for instance during the next intense drainage season. Consequently, the forest buffer received epoxiconazole loads during the intense drainage season of the three years ranging from 19 mg to 1.4 g. Opening the buffer zones while drainage starts again may be an efficient strategy to catch large loads.

#### *3.2.1.c Nitrate and suspended sediments*

Overall, 46 and 12 % of watershed outlet nitrate loads did cross the artificial wetland and the forest buffer, respectively. In addition, these values were 39 % (artificial wetland) and 6 % (forest buffer) for suspended sediments. Roughly, major nitrate loads entered the wetland during the end of drainage periods whereas the forest buffer did catch nitrate loads in drainage initiation periods.

### 3.2.2 Presence of the buffer zones: weighting the impacts

From the 2007 – 2010 mass balance, it appeared that 25 % of the watershed outlet volumes were not given back to the stream (Table IV-2 and Fig. IV-12). It is arguable whether such water losses should be considered or not as an issue. Indeed, it implies that lower water volumes reached the stream compared to a situation where no buffer zones would have been set up. The “Le Calais” creek, located at the Bray watershed outlet, is permanent and does not suffer from extended periods of low flows. However, the overall hydrologic cycle was modified by the buffer zones which increased water losses by evapotranspiration and possible vertical downward leaks. However, such site-specific consequences on receiving waters' hydrology need to be seriously considered while implementing buffer zones.

Compare to watershed outlet loads, the presence of the buffer zones did help reduce pesticide loads and therefore exhibited a positive impact on downstream water quality. Indeed, for the most frequently used and quantified pesticides, load reductions were 10 % for isoproturon, 21 % for epoxiconazole, 32 % for metazachlor, 55 % for diflufenican and 63 % for chlorotoluron.

## 4 Conclusions

All pesticides altogether, on average, the artificial wetland and the forest buffer provided a dissipation of 39 % of watershed outlet pesticide loads. This gain is important and confirms that the presence of the buffer zones helped decrease pollution to the downstream creek. Overall, most pesticide loads entered the buffer zones during drainage initiation or end of drainage seasons. However, as noted previously, when large flows start again after a period of low flow, high pesticide loads may be exported and caught by the systems. Pesticide concentration and load reductions were widely variable. There were uncertainties on estimated loads were based on low concentrations and accounted for only few samples whose concentrations were larger than the limit of quantification. Despite a lower number of data than for the artificial wetland, the forest buffer had lower water volume losses than the artificial wetland and seemed to perform well for pesticide concentration and load reductions.

Desorption of some previously sorbed molecules, as observed in the laboratory and on-site tracer experiments, was also suspected at this large time and space scales. For weakly sorbing molecules associated with large application rates, like isoproturon, reducing application rates, in complement to buffer zones implementation may be needed to reach significant improvement on downstream water quality.

The Bray artificial wetland may be under-sized to accommodate watershed outlet pesticide concentrations and provide an optimized treatment. Degradation was unlikely to have occurred to a large extent due to limited residence time. However, water level fluctuated thus implying alternation between aerated and anoxic conditions leading to complementary microbial processes. Bray water level was usually lower than 40 cm in the first artificial wetland and 10 cm for the third cell. Increasing water volume capacity by digging deeper the third wetland cell may provide larger pollutant removal due to increased storage capacity and residence time. Based on the Bray demonstration site results and literature studies, the concluding chapter will overview proposals to attain larger pesticide pollution mitigation.

# GENERAL CONCLUSION

# 1 Scientific conclusions

## *1.1 Pesticide mitigation potential exists in buffer zones*

Three years of data were collected on-site for an artificial wetland and a forest buffer. However, because of more frequent closures, a lower number of data was gathered for the latter. Thanks to the multi-scale approach employed in this study, it could be concluded that potential at reducing pesticide pollution exists in artificial wetlands and forest buffers. The "black box" field-scale analysis showed that the presence of the buffer zones positively affected water quality.

The sampling strategy was not perfectly adequate to assess concentration reduction for such tile-drained watersheds presenting continuous but variable outflows. Nevertheless, the most representative results highlighted that some concentration reductions did occur depending on molecules. Overall, concentration peak attenuation can be expected through buffer zones. However, in some occasions, outlet concentrations were larger than those at the inlet. Artificial wetlands and forest buffers are not "pesticide production" sites. Such results may therefore be explained by delayed transfer of pesticides through the systems, particularly for those continuously entering the buffer zones, like isoproturon. It can also possibly be due to desorption of previously adsorbed molecules.

Contrary to concentration reduction assessment, the sampling strategy was well adapted to large time-scale mass balance calculations. It allowed determining the gain that buffer zone implementation represented for the receiving stream, as well as possible negative retroactions it could have generated. On average, 39 % of watershed outlet pesticide loads did not reach the downstream creek thus undoubtedly improving its water quality. This positive effect might be partly counter-balanced by buffer zone impacts on water quantity. Forcing watershed outlet flow to cross the artificial wetland and the forest buffer also resulted in the loss of 25 % of the water volumes, through infiltration and evapotranspiration. This was not considered as an issue at the Bray catchment. However, this site-specific aspect may be taken into consideration to determine whether reducing water flows may have a negative or negligible effect on downstream water bodies. In large watersheds including several constructed buffer zones, water volume reductions from headwater sub-watersheds to lower parts of the watershed may negatively affect the whole catchment hydrology by cumulating water losses little by little.

## *1.2 Understanding pesticide fate in buffer zones*

### 1.2.1 Adsorption – desorption

Thanks to laboratory and tracer experiments, adsorption and desorption were found to be key processes governing pesticide fate in such systems. Adsorption was observed on on-site naturally present substrates like wetland sediments, forest soil and litter, as well as on man-introduced (or naturally colonizing) substrates like wetland plants. Adsorption corresponds to the transfer of pesticides from one phase (water column) to another (solid sorbent). It has both positive and negative impacts (Table C 1). First, adsorption may be wished because it delays pesticide transfer towards receiving surface waters and it attenuates concentration peaks. From an acute ecotoxicity perspective this can be considered as a first positive impact. However, a question remains concerning strongly bound pesticides to wetland sediments or forest soil. The "sink" potential of buffer zones for pesticides may result in their accumulation. Could these buffer zones further become pesticide "source" through release of adsorbed molecules? And should that be a concern? Further studies should focus on

pesticide concentration evolution in the sediment and vegetal compartments of buffer zones. The punctual analysis that was carried out at Bray was not exhaustive but did not show any accumulation of pesticides in wetland sediments and forest soil. Laboratory experiments and tracer experiments confirmed that adsorption could be a reversible process, whose importance depends on substrates and molecules. Desorption of previously sorbed molecules was found to occur particularly for moderately sorbing pesticides like isoproturon and metazachlor. Desorption may be seen as a process increasing pesticide availability for microorganisms thus making them more available to degradation provided contact between molecules and microorganisms occurs. However, if desorbed molecules do not undergo either degradation or new adsorption processes, they may infiltrate or reach surface waters. This may explain the occurrence of larger outlet than inlet concentrations, as particularly observed at the end of tracer experiments, or suggested from field-scale assessment.

| | **Adsorption** | **Desorption** |
|---|---|---|
| **Positive impacts** | - Delays pesticide transfer.<br>- Dampen concentration peak. | - Make pesticides available to microorganisms for degradation. |
| **Negative impacts** | - Leads to pesticide accumulation in sediments. | - Return pesticides to ground or surface waters. |

**Table C 1: Counter-balanced effects of adsorption and desorption processes.**

### 1.2.2 Degradation

Studying epoxiconazole disappearance at laboratory scale was almost a “worst-case” scenario as its half-life is known to be of several months from agricultural soils or rarely published water/sediment studies. However, epoxiconazole degradation was demonstrated as attested by the generation of metabolites. Nevertheless, mineralization was limited to less than 5 % even after 177 days of incubation. Degradation is clearly variable from one molecule to another. The 66.5-h calculated hydraulic residence time ($\tau$) under low flow conditions may not have been large enough for degradation to occur to a large extent, given that pesticide half-lives are frequently longer than 2 days. However, the pesticide residence time (PRT) is supposed to be longer than $\tau$ because of transfer delays due to molecule adsorption on buffer zone substrates. Focusing on a limited number of parent molecules but in conjunction with their main metabolites at both buffer zones inlet and outlet may help determining if degradation is happening under field conditions. Characterizing parent molecule and metabolite toxicity should also be implemented to assess expected impacts on receiving ecosystems. Pesticide degradation is frequently seen as a detoxification process generating molecules of lower toxicity than parent molecules. Even if this is probably mostly true, degradation products may also present high, even if lower, toxicity and may be harmful to aquatic systems. The analytical method developed for this work did not allow for metabolites characterization. Ecotoxicological effects were not characterized either. Such studies may be interesting complementary approaches to the present analysis.

## 2 Derived operational conclusions

Buffer zones like artificial wetlands and forest buffers can be considered as “ecological engineering systems”. Indeed, their creation or restoration combines human needs, through the water quality improvement objective, and ecosystem functions. Starting guidelines are proposed below to implement buffer zones in the landscape and optimize their design considering pesticide pollution reduction as the driving objective.

*2.1 Characterizing watershed outflows to select location and inlet functioning strategy*

Depending on land availability and watershed outlet volumes, buffer zones may be placed either in-stream ("in series") or off-stream ("in parallel"). The Bray watershed (46 ha) generated too large volumes to be all treated by the systems. The forest buffer and the artificial wetland were therefore placed "in parallel" to the main agricultural ditch. When possible, buffer zones should be implemented closer to pollution source, in smaller watershed so as human intervention is reduced with in-stream located systems. If a strategy has to be implemented to select flows of most concern for pesticide transfer, by-pass structures must be set up to divert uncaught volumes to downstream waters. They can be either self-functioning or require human intervention. The open – close strategy implemented at Bray did work well at catching pesticide loads. However, this would imply a larger involvement of the land-owner often being the farmer, which is not a step in the right direction according to ecological engineering recommendations. The selected strategy proposed to the farmer was to open the buffer zones right after its applications and close them approximately one month later. This proposal was undertaken for its simplicity and reduced farmer intervention on-site but may have led to missing large pesticide loads. More research on on-site buffer zone functioning may help refine guidelines to select and implement a robust selection of flows of most concerns. Eco-technologies should be designed so that human intervention is reduced as much as possible. Watershed managers should be aware that sociological issues may also arise from required increased land-owner intervention.

The assessment of watershed outlet flows and pesticide loads timing clearly showed that the first flows following applications were among those presenting the highest loads. However, it was also highlighted that large pesticide loads were also measured while watershed outflows resumed after an antecedent period of low or no flows. An easily available parameter from which watershed outflows may be anticipated by land-owners (i.e. buffer zone managers) is rain predictions. For small buffer zones like those implemented at Bray, focusing on the first flows of the drainage initiation season is definitely recommended as it is easy to implement. Indeed, the management will consist in opening the systems in summer and waiting for the first flows to occur. Deciding when to close buffer zones to prevent additional flows to enter, and give time to caught volumes to undergo dissipation processes, is much more challenging. It was shown that in 2008 – 2009, most isoproturon loads were missed because watershed outflows occurred later than expected.

As noted in this dissertation but not assessed, an open – close strategy, or simply enabling water level to fluctuate in buffer zones is likely to lead to alternation between aerobic and anoxic conditions. Oxidized conditions are frequently associated with larger microbial activity and pesticide degradation rates. A better characterization of the degradation of the pesticides of interest on buffer zone substrates under oxidized conditions could supplement the laboratory experiments carried out under flooded conditions.

*2.2 Buffer zone inlet design*

A proper design of wetland or forest buffer inlet structures is required to ensure efficient treatment. Despite a lower number of samples in the forest buffer located at Bray, it showed very promising results at reducing pesticide pollution. As for the artificial wetland, adsorption was found to be a key parameter on both forest soil and litter. However, desorption was also found from both laboratory and tracer experiments. At Bray, the forest inlet seemed very efficient to convert watershed outlet channelized water flows into distributed sheet-flow. It demonstrates that not only runoff pollution can be dissipated through vegetated zones, but drainage as well, provided such a flow distribution conversion has been done. The present design may be recommended for tile-drained canalized flows. It consisted in creating an inlet

distributing ditch along the buffer zone width from which water could overflow in a fairly evenly distributed runoff. Depending on buffer soil microtopography, designers may need to level off forest buffer soil and create multiple incisions in the inlet ditch to help water distribute.

A buffer zone including water storage (as in the artificial wetland) may be better designed with widened inlet to ensure inlet flow rate reduction. This may help reduce peak discharges and thus increase wetland hydraulic buffer function. Sedimentation may be enhanced provided the inlet also consists of a deep pool where sediments can accumulate and are not prone to resuspension. That might be done at Bray to help decrease pesticide-laden sediments transfer through the system.

Diameter limited inlet structures associated with by-pass structures may be a strategy to control inlet flow rates. This should be adapted to catchment and buffer zone sizes. At Bray, 56 % of watershed outlet peak flow rates were lower than 35 L/s (0.8 L/s/ha, maximal inlet flow rate controlled by 200 mm diameter inlet PVC pipe). Wetland was suspected to be undersized for watershed outlet volumes and pesticide loads, despite being able to catch and reduce significant pesticide loads. At Bray, available land area was entirely utilized to implement the artificial wetland. Apart from increasing its volume, another option may be to pay more attention to flows of most concerns that should be primarily treated.

*2.3 Residence time: a fundamental parameter to enhance dissipation*

The discussion about epoxiconazole degradation, the fact that the identified key processes at the field-scale were probably adsorption – desorption, and results from field scale buffer zones argue for residence time being a key parameter to improve water quality. This parameter may be partly controlled. Giving time to pesticide molecules to interact with major substrates or microorganisms is expected to enhance pesticide removal.

Increasing residence time can be done through lengthening water flow path. This could be achieved with **dams** in order to force water to take a more tortuous path. At Bray, there are few chances this could be implemented as dams already exist. However, implementing additional obstructions in strategic locations where water velocities are fast might be thought of. While placing inlet and outlet structures, it should be ensured that they are in **opposite locations** to avoid short-circuits.

In addition, the larger the wetland water storage capacity, the longer the residence time. This can be tackled adapting wetland size and volume. Increasing **buffer zone surface area** may be discussed with land-owners and can be considered one of the biggest constraints not fully controllable by wetland managers. The closer the buffer zones to pollution source, the lower these constraints and the smaller the system size. At Bray, the ratio between each buffer zone surface area to that of the watershed was approximately 0.4 %. The systems appeared to be under-sized given the watershed outlet pollutant load produced every year, despite the selection of flows. Consequently, a ratio of 1 or 2 % may be a better criterion to achieve significant reduction.

In addition to surface area, **wetland volume** has to be optimized. It is however usually recommended not to dig deeper than 80 cm to ensure light penetration and possible pollutant photodegradation, as well as to provide a suitable environment for vegetation growth. However, digging deeper pools (e.g. 1 m depth) spread out in wetland systems may provide additional water storage capacity. As part of ecological engineering approach, besides the first treatment objective, additional positive effects of buffer zones may be wanted. For instance, variable depths resulting in the alternation of deep pools and shallow reservoirs can provide both water quality improvements and enhanced biodiversity. Ecological engineering

principles required that systems (e.g. wetlands) should be designed for multiple objectives but keeping one main objective as a starting point. In the present case, water quality improvement is the starting aim but biodiversity increase, landscape aesthetics, educational objectives were also targeted at Bray. According to water depth, different plant and animal species will colonize the systems. In addition, deep pool at wetland inlets are often recommended to reduce water flow velocities, increase residence time and favour sedimentation. The ratio between the Bray artificial wetland volume (330 $m^3$) and watershed surface area (46 ha) is approximately 7 $m^3$/ha. Another wetland system (Aulnoy, Seine-et-Marne, France) monitored by the Cemagref and briefly studied in the laboratory experiment has a much larger volume due to the presence of a deep storage reservoir. The ratio of this system is of 300 $m^3$/ ha. Very low pesticide concentrations were recorded at the outlet which was in part explained by dilution. From a water quality perspective, this system may be much too large to ensure sufficient contact between substrates and pesticides for their adsorption and degradation. Consequently, an intermediate value ranging from 15 to 20 $m^3$/ha may be a starting design criteria to adapt to on-site constraints. At Bray, increasing the third artificial wetland cell depth may result in increased residence time and water treatment. In addition, suspended sediments were not reduced to a large extent which may be due to a lack of deep pools and wide inlet to reduce flow velocity or particle resuspension in shallower zones during large flow events, associated with faster water velocities.

*2.4 Vegetation*

Introducing vegetation has been suggested in this study. Plants are one of wetland components that can at least be partly controlled by wetland managers. It is often recommended to use native species so as unwanted and uncontrolled effects like invasion be less likely to happen. Vegetation has direct and indirect effect on pesticide pollution reduction. Well distributed, it helps reduce water velocity thus increasing residence time. At Bray, carrying out a new tracer experiment under similar flow conditions as those from the March 2008 experiment would be interesting to compare hydraulic metrics while vegetation has now colonized most of the system.

From laboratory experiments, wetland plants demonstrated they can enhance adsorption of molecules. This is an additional temporary sink to wetland sediments even if desorption appeared to be easier from plants than sediments. Wetland plants were also associated with epoxiconazole degradation and non-extractable residue formation thus playing a role to reduce pesticide pollution from the water column. In addition, plants can locally aerate water and sediments, at stems and roots proximity. This implies that not only anoxic processes can take place but aerobic degradation as well. The latter frequently showed faster rates than anoxic biodegradation.

It is important to note that such high potential attributed to wetland plants requires interaction between pesticides and plant tissues for it to be exhibited. Apart from suggesting increasing vegetation density, this comment leads us back to wetland hydrology. Indeed, short-circuits are to be avoided to ensure that contact between pollutant and wetland substrates may occur.

In the forest buffer, not only the forest soil exhibited a role in pesticide retention and degradation. Accumulated litter through which water runs off enhanced pesticide sorption, as suggested from laboratory experiments. Pesticide adsorption and desorption from litter seemed to be slightly lower than those measured on "fresh organic matter" like wetland plants. Laboratory results also showed an increased presence of non-extractable residues of epoxiconazole in forest litter and little mineralization or metabolite formation. As for wetland sediments or forest soil, the possible long-term accumulation of pesticides in this compartment should be characterized.

*2.5 Substrate addition and microbial inoculation*

The addition of exogenous substrates in buffer zones may be considered as a possible improving measures leading to more engineered systems. This was not specifically tested in the present work. However, given the results from laboratory experiments on vegetal substrates (wetland plants and forest litter), it can be suggested that the addition of organic material into buffer zones may enhance pesticide mitigation.

Such engineered proposals should however be done with cautions and their possible positive or negative retroactions must be anticipated. For instance, the addition of organic substrates, under the form of a straw ball inside the wetland may lead to stagnant zones in water that could attract tiger mosquitoes carrying Aedengue angerous diseases contaminating wild deers drinking in the wetland and hunted by farmers that may thus suffer from the transmitted disease and…. Maybe (hopefully!) not. The “Butterfly effect” translates that, small variations in a system initial state (straw ball addition) may generate large variations (population affected by a disease) in the long-term behaviour of a dynamic system (wetland).

One of ecological engineering principle is to avoid over-engineering created systems like artificial wetlands and give time to systems to establish themselves. However, pesticide pollution reduction is challenging and may require more research and design optimization guidelines to help buffer zones express their potential. A trade-off between designing “natural” and “engineered” systems must be found.

Modelling approaches may be needed to test several scenarios and their possible impacts on the environment. Instead of over-engineering a local system, a key approach would be to “think global”. For a water quality improvement perspective, buffer zones should be part of an integrated strategy as it was shown that they can not be considered the "miracle solution" to completely remove pesticide pollution. This should be defined considering large space and time scales. Pesticide application rate reductions, application date shifting, use of existing landscape elements (forests, shrubs, grassed zones, wetlands or ponds) and creation of new ecological solutions must be considered as complementary mitigation measures.

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# APPENDICES

## Appendix I Scientific studies dealing with wetlands and pesticides (on-site systems)

| Ref | Pesticides | CW | Study site location | Water-shed area | Hydrology | Soil | Vegetation | Results |
|---|---|---|---|---|---|---|---|---|
| Blankenberg et al. 2007 | metalaxyl<br><br>metamitron<br>metribuzin<br>propachlor<br>linuron<br>fenpropimorph | | Norway | | | | | Mass retention: 14 (2003) - 23 (2004) %<br><br>42 - 28 %<br>11 - 19 %<br>37 - 32 %<br>26 - 56 %<br>27 - 50 %<br><br>**Engineering:** testing of different media: flagstones and straw = highest total pesticide retention. Average retention for both years = 15 - 41 %. Low solubility ==> highest mass retention (linuron + fenpropimorph) but < 50 %; lowest retention for the most soluble (metalaxyl) |
| Alvord and Kadlec 1996 | atrazine | 3 CW | IL, USA | | HRT = 8 d for 2 CW and > 8 d for the 3rd | | | Peaks (max = 3 µg/L) were delayed, reduced, spread out; the lowest hydraulic loading rate (250 $m^3$/d) was associated with the highest detention time (51.2 d) and atrazine mass removal (64 %). For the other 2 wetlands (HRT = 8 d approx) ==> 26 and 33 % removal and HLR = 1220 and 1560 m3/d |
| Conkle et al. 2008 | cotinine<br>caffeine<br>CBZ<br>other pharmaceuticals ... | | | | | | | varying concentration reduction (-202 % to 77 % calculated) for the sole wetland; overall WWTP efficiency = good |

| Ref | Pesticides | CW | Study site location | Water-shed area | Hydrology | Soil | Vegetation | Results |
|---|---|---|---|---|---|---|---|---|
| Cheng et al. 2002 | parathion<br>omethoate<br>4-chloro-2-methyl-phenoxyacetic acid (MCPA)<br>dicamba | **mesocosms** 1 m² each CW (two CW) | ? | | | | | mass removal after 4 months = 100 %<br>100%<br>36%<br>0%<br>Mesocosms study; |
| Fairchild et al. 2002 | metribuzin | 10 mesocosms of 0.1 ha each (750 m3; max depth = 2 m) macrophyte, fish | Columbia, MO (missouri), USA | | | | | $DT_{50}$ = 5 days; no effect on plants or direct effect on fish for 5 concentrations : 0 - 9 - 19 - 38 - 75 µg/L |
| Huang et al. 2006 | diuron<br>isoxaben<br>oryzalin | lab expe - engg - shredded cedar mulch | | | | | | batch + column expe ==> mulch usuful for pesticide runoff control |
| Hunt et al. 2008 | chlorpyrifos<br>diazinon<br>dioxathion<br>oxadiazon<br>pyrehroids<br>organochlorines<br>dimethoate<br>ethion<br>malathion<br>thionazin<br>carbaryl<br>carbofuran | VTS (= vegetated treatment system) pond; #1: 0.15 ha, 0.3 % WS area; 640 m3; et #2: 0.2 ha, 0.57 % WS area; 1350 m3 | | | #1: HRT = 7.4 d et aveQ=1 L/s et #2: HRT=2.3 d; aveQ=6.8 L/s | | | 55% concentration change in VTS2 pond<br>-10 % (VTS2)<br>20 % (VTS2)<br>40 (VTS2)<br>65 (VTS2) et -15 (VTS1)<br>85 (VTS2) et 20 (VTS1)<br>20 % (VTS1)<br>60 (VTS1)<br>40 (VTS1)<br>95 (VTS1)<br>5 (VTS1)<br>95 (VTS1) |

| Ref | Pesticides | CW | Study site location | Water-shed area | Hydrology | Soil | Vegetation | Results |
|---|---|---|---|---|---|---|---|---|
| Kidmose et al. 2010 | isoproturon | | Denmark, EU | | inflow = GW | | | 2/3 of injected IPU can be removed (tracer expe); IPU retarded by a factor of 2 to 4 probably due to high OM of peat; DT50 = 12 to 80 days; aerobically degraded; |
| Llorens et al. 2009 | Pharmaceuticals and personal care products PPCPs | Surface Flow CW = 1 cell = 1 ha, water depth from 0.3 to 1.5 m; includes an island(=550 m²); max L*l=189*53m | | industrial + urban watershed | 1st period= 100m3/d; HRT=1 month; HLR=1 cm/d 2nd period/= 250 m3/d; HRT=12.4 d; HLR=2.5 cm/d | | phragmites australis + typha latifolia | Usually, removal rates > 70% except for clofibric acid (34%) and carbamazepine (39%); higher removal rates for higher HRT apparently (when they compare their results to litt) |
| Matamoros et al. 2007 | lindane<br>pentachlorophenol<br>endosulfan<br>pentachlorobenzene<br>alachlor<br>chlorpyrifos<br>mecoprop<br>simazine<br>clofibric acid<br>diuron | **HSSF** CW; 0.3 m water depth on average; 55 m² surf area; | Catalonia, Spain, EU | | HLR = 36 mm/d | | | Removal % > 90 % (highly removed)<br>80 - 90 % (efficiently removed)<br>20 % (poorly removed)<br>recalcitrant to elimination<br>**Enginnering:** Poor accumulation in the gravel bed (0 - 20 %); processes may be plant uptake + biodegradation |
| Matamoros et al. 2008 | 3 pesticides (see below) & several PPCP<br>mecoprop<br>mcpa<br>terbutylazine | Surface Flow CW = 1 cell = 1 ha, water depth from 0.3 to 1.5 m; includes an island(=550 | | industrial + urban watershed | 100m$^3$/d; HRT=1 month; | | phragmites australis + typha latifolia | Overall > 85% except for clofibric acid and carbamazepine; slightly better in June than Feb ==> seasnal trend for mol with low biodegraation and moderate phtodegradation potentials (naproxen and diclofenac)<br>inlet conc=7.8 µg/L; removal June=79%; removal feb=91%<br>2.01 µg/L; 93 % ; 79 %<br>2.30 µg/L; 1 %;80% |

| Ref | Pesticides | CW | Study site location | Water-shed area | Hydrology | Soil | Vegetation | Results |
|---|---|---|---|---|---|---|---|---|
| | | $m^2$); max L*l=189*53m | | | | | | |
| Machate et al. 1996 | phenanthrene | **subsurface flow** 5 steel tanks in cascade = 2.5*2.3*1.2; 1m height coarsely graded lava material; | | | subsurface flow | | typha spp. and scirpus lacustris | > 99.9 % removal; microbial degradation suspected (b/c metabolite apparition); **engineering:** lava materisl = important role as a support matrix for bacteria growth; temperature plays a role in phenanthrene adsorption adsorption; most of the removal took place in the 1st (out of 5) tank = 98%; slightly more in the second and then, no more phenanthrene |
| Miller et al. 2002 | atrazine<br>alachlor<br>total = 9 pesticides dont les deux précédents où ils ont aussi regardé les diff par saison | 177 $m^2$; 56 m3; 0-1m ; surface flow; data from Dec. 1993 to Dec. 1997 | IL, USA | 10.9 ha | HLR = 15.58 cm/d | org matter, decomposing and 50 cm deep sediment layer | duckweed (lemna spp) | Spring, fall, winter: no removal < LQ; Summer: ATZ in < out mais n.s.<br>Spring, summer, fall, winter: no removal; < LQ;<br><br>For 9 pesticides, mass reduction varied from -215.2 to 96.1 % but the only one signif = -215.2 (ethalfluralin) !! but it is biaised because most of concentration values are on average < LQ |
| Moore et al. 2000 | atrazine | 8 cells: 2 Cin = 73 µg/L; 2 Cin = 147 µg/L, 1=control = 0µg/L and 3 as water source; 59-73*14*0.3 m | MS, USA | simulation of RF = 2.54 mm on 4, 40, 400 ha | | 1.6% OM | | Cin = 73 ==> 66-70 % removal (16-21 d DT50) after 35 days; Cin = 147 µg/L ==> 34-37% (46-48 d); for an outlet concentration obj = 20 µg/L ==> 101-164 m needed for Cin=73 µg/L and 103-281 m needed for 147 µg/L |
| Moore et al. 2001 | metolachlor | | | | | | | Cin = 73 ==> 91% removal (DT50: 13 d in water and 17 d in plants) after 35 days, avec 16% ds les plantes; Cin = 147 µg/L ==> 87% (DT50: 8 d in water and 61 d in plants); for an outlet concentration obj = 44 µg/L ==> 102-170 m needed for Cin=73 µg/L and 100-400 m needed for 147 µg/L |
| Moore et al. 2002 | chlorpyrifos | | | | | | | After 84 days: Cin=147 µg/L: 99% removed from water, 44-89% from sed and 89-97 % from plants; DT50water=13 days<br>Cin=73 µg/L: 99 % (water), 94 % (sediment), 97 % (plants); DT50water=4.6 days Cin = 733 µg/L: 99 % (water), |

| Ref | Pesticides | CW | Study site location | Water-shed area | Hydrology | Soil | Vegetation | Results |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 50%(sed), 94% (plant): all combined together: >83% removal ==> approx: overall: 55 %(sed), 25 %(plant), > 90 % (water) |
| Moore et al. 2009 | lambda-cyhalothrin<br>cyfluthrin | | | | | | | water / sed / plant % for Conc: 3 / 63 / 34 & for Mass: 6.3 / 47 / 49; proposed wetland length=217 m<br>water / sed / plant % for Conc: 4 / 2 / 94 & for Mass: 18 / 7 / 75proposed wetland length=210 m<br>==> good removal from water b/c of association to sed or plants |
| Poissant et al. 2008 | atrazine | | | | | | | was degraded; more info about transfer from watershed to wetland |
| Rose et al. 2006 | fluometuron<br>diuron<br>aldicarb<br>endosulfan | 2 cells in a series: (1) open-water 0.5m depth / 100 m² and (2) vegetated 1m depeth / 200m² | Australia | 30 ha cotton field | | | | 1st year: 0-34%open = veg (statistically); 2nd year: veg (58%) > open (41%)<br>1st year: 27-55% open = veg (statistically)<br>1st year: 15-39 %open = veg (statistically)<br>2nd year: 24 % open et 27 % veg |
| Runes et al. 2003 | atrazine | 5 sequential cells 3 * 40 m | OR, USA | 2.4 ha nursery land runoff | different expe simulating runoff with varied Frequencies and Intensities | 2.13 % OC; pH=6.03; 10%sand; 20%clay; 70%silt | > 75 % typha latifolia | HUGE inlet concentration = 400 mg/L In 1998: %mass removal varied from 69 to 98.7 % except one value for cell 1 = 3% for different cells and experiments; In 1999: -6 to 84; worst case <=> high runoff freq + intensity + amount ==> compromised treatment; apart from this case, no diff in eff b/w expe ; sorption = primary mechanism |
| Schulz et al. 2001 | azinphos-CH3<br>chlorpyrifos<br>endosulfan | 0.44 ha CW (134 * 36 m * 0.3-1) | | 43 ha (orchard, pasture, forest) | | | no veg in the 1st 30 m; 60%typha; 10% juncus 5% cyperus | Cin=0.85 µg/L ==> 77 - 93 %<br>Cin = 0.02 µg/L ==> < LD at outlet<br>Cin = 0.2 µg/L ==> < LD at outlet<br>increased amount of pesticides in the sediments within the 5 months ; the problem of lack of water storage capacity is highlighted (small size) |

| Ref | Pesticides | CW | Study site location | Water-shed area | Hydrology | Soil | Vegetation | Results |
|---|---|---|---|---|---|---|---|---|
| Keefe et al. 2004 | volatile organic compounds | 4 individual CW 0.9 - 1.34 ha; ils ne parlent que de H1 | AZ, USA | | total pour les 4 CW= 3780 m3/s (!!) | | | Removal : 63 - 87 % for TARGET VOC et 20 à 59 % pour low-level VOC |
| Haarsad and Braskerud 2005 | propachlor<br>linuron<br>metamitron<br>propiconazole<br>fenpropimorph<br>metribuzin<br>metalaxyl | 840 m², sedimentation pond(0.5m)+ 3 CW (0-0.3m) + 3 Sfzones (0 m )+ 1 wetland filter | Norway, EU | 22 ha | | 18-37 % organic sediments | | 2000: G1(31) G2(67) ; 2001 G1(-4) G2(14)<br>2000: G1(18) G2(30) ; 2001 G1(0) G2(3)<br>2000: G1(23) G2(58) ; 2001 G1(-18) G2(7)<br>2000: G1(16) G2(25) ; 2001 G1(5) G2(13)<br>2000: G1(24) G2(36) ; 2001 G1(2) G2(10)<br>2000: G1(22) G2(40) ; 2001 G1(15) G2(19)<br>2000: G1(12) G2(41) ; 2001 G1(-6) G2(-11)<br>It represents < 2 % of applied pesticides; sometimes outlet conc still high enough to be potentially harmful to aquatic life; high concentrations = high reductions; increase electrical conductivity = increase conc reduction; doubling CW surface area ==> increase reduction |
| Braskerud and Haarsad 2003 | propachlor<br>linuron<br>metamitron<br>propiconazole<br>fenpropimorph<br>metribuzin<br>metalaxyl<br>mecoprop<br>dicamba<br>MCPA<br>dichlorprop<br>bentazone<br>fluroxypyr | 840 m², sedimentation pond(0.5m)+ 3 CW (0-0.3m) + 3 Sfzones (0 m )+ 1 wetland filter | Norway, EU | 22 ha | HLR = 0.8 m/d | 18-37 % organic sediments | | 67 % (2000) & 14 % (2001)<br>30 % (2000) & 3 % (2001)<br>58 % (2000) & 7 % (2001)<br>25 % (2000) & 13 % (2001)<br>36 % (2000) & 10 % (2001)<br>40 % (2000) & 19 % (2001)<br>41 % (2000) & -11 % (2001)<br>23 % (2001)<br>3 % (2001)<br>27 % (2001)<br>35 % (2001)<br>2 % (2001)<br>-2 % (2001)<br>increase electrical conductivity = increase conc reduction; |

| Ref | Pesticides | CW | Study site location | Water-shed area | Hydrology | Soil | Vegetation | Results |
|---|---|---|---|---|---|---|---|---|
| Schulz et al. 2001 (conc, load, toxicity) | azinphos-CH3 | 0.44 ha CW (134 * 36 m * 0.3-1) | | 43 ha (orchard, pasture, forest) | | | no veg in the 1st 30 m; 60%typha; 10% juncus 5% cyperus | 54.1 % retention of load et 90.8 % concentration reduction |
| Schulz et al. 2003 | azinphos-CH3 | 0.44 ha CW (134 * 36 m * 0.3-1) | | 43 ha (orchard, pasture, forest) | | | no veg in the 1st 30 m; 60%typha; 10% juncus 5% cyperus | 60.5 % retention of load and 90.1 % concentration reduction |
| Sherrard et al. 2004 | chlorothalonil chlorpyrifos | 4 CW : 185 * 63 *63m | | | HRT = 72 h | 8.7 % OM | 400 shoots /m²scirpus cyperinus | concentrations declined to < 0.1 µg/L<br>> 98 % concentration reduction<br>simulated experiments - very high Cin (256/292/308/329 µg/L expe # 4 for instance) |
| Stearman et al. 2003 | simazine<br>metolachlor | 14 CW cells: 1.2*4.9 or 2.4*4.9 and 30-45 cm deep | TN, USA | 465 m² ; runoff from container nurseries | 3 flows: 0.24, 0.12 or 0.06 m3/d | | 50 % cells were vegetated with Scirpus validus | with plants 77.1 % (SMZ) > without plants 64.3 % (SMZ)<br>with plants 82.4 % (MTL) > without plants 63.2 % (MTL)<br>Slower flow rate = slower HRT & lower mass loading ==> higher removal<br>lowest flow rate & lowest mass loading ("ideal" cond) ==> 90.2 % (MTL) & 83 % (SMZ)<br>microbial degradation may be limiting step compared with pesticide sorption |
| Budd et al. 2009 | pyrethroid and organophosphorus pesticides<br>bifenthrin | 1 sediment basin + 2 CWs: CW1 = 2 cells totalling 450 | CA, USA | 450 ha | HRT CW1 = 1h; CW2 = 18h | highly permeable in the wetland vicinity | main segment of CW1 = no vegetation ; then, densely vegetated | Pyrethroids are likely to be primarily associated with fine & light particles; whereas, sedimentation first affect heavy (sand) suspended particles<br>Concentration reduction CW1: 69 %; CW2 : 84 % Load reduction CW1 : 98 % ; CW2 : 95 % |

| Ref | Pesticides | CW | Study site location | Water-shed area | Hydrology | Soil | Vegetation | Results |
|---|---|---|---|---|---|---|---|---|
| | cyhalothrin | m flow length; CW2 = 1 cell of 720 m flow length | | | | | | Concentration reduction CW1: 71 %; CW2 : 90 % Load reduction CW1 : 98 % ; CW2 : 99 % |
| | cypermethrin | | | | | | | Concentration reduction CW1: 52 %; CW2 : 64 % Load reduction CW1 : 97 % ; CW2 : 95 % |
| | esfenvalerate | | | | | | | Concentration reduction CW1: 87 %; CW2 : 77 % Load reduction CW1 : 100 % ; CW2 : 99 % |
| | permethrin | | | | | | | Concentration reduction CW1: 90 %; CW2 : 94 % Load reduction CW1 : 100 % ; CW2 : 99 % |
| | chlorpyrifos | | | | | | | Concentration reduction CW1: 61 %; CW2 : 52 % Load reduction CW1 : 98 % ; CW2 : 93 % |
| | diazinon | | | | | | | Concentration reduction CW1: 22 %; CW2 : 82 % Load reduction CW1 : 92 % ; CW2 : 68 % |

**Table A 1: Summary of literature about pesticide removal in field-scale wetland systems.**

## Appendix II Scientific papers dealing with nitrate removal in wetlands

| Year | Authors | Number of wetland system | Wetland identification | Type Nat vs. CW | Flow regime FWS vs. SSF | Conc in mg/L | Conc out mg/L | Load in mgN/ $m^2$/yr | Load out mgN/ $m^2$/yr | Nitrate removal conc % | Nitrate removal load % | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1994 | Phipps and Crumpton | 1 | EW3 1991 Apr-Nov | CW | | | | 21.6 | 4.7 | NA | 78 | |
| | | 1 | EW4 1991 Apr-Nov | CW | | | | 3.2 | 0.2 | NA | 95 | |
| | | 1 | EW5 1991 Apr-Nov | CW | | | | 20.2 | 3.2 | NA | 84 | |
| 1995 | Thomas et al. | 1 | *schoenopletus validus* | CW | | 20 | 7 | | | 65 | | |
| | | 1 | *juncus ingens* | CW | | 20 | 4 | | | 80 | | |
| | | 1 | mixed species | CW | | 20 | 6 | | | 70 | | |
| | | 1 | unvegetated | CW | | 20 | 5 | | | 75 | | |
| 1994 | Hey et al. | 1 | EW3 year 1990 | CW | | 1.87 | 0.54 | 1388 | 286 | 71 | 79.4 | |
| | | 1 | EW4 1990 | CW | | 1.87 | 0.24 | 369 | 225 | 87 | 39 | |
| | | 1 | EW5 1990 | CW | | 1.87 | 0.53 | 1015 | 206 | 72 | 79.7 | |
| | | 1 | EW6 1990 | CW | | 1.87 | 0.32 | 691 | 8 | 83 | 98.8 | |
| | | 1 | EW3 year 1991 | CW | | 1.22 | 0.23 | 352 | 51 | 81 | 85.5 | |
| | | 1 | EW4 1991 | CW | | 1.22 | 0.1 | 87 | 4 | 92 | 95.4 | |
| | | 1 | EW5 1991 | CW | | 1.22 | 0.18 | 271 | 21 | 85 | 92.3 | |
| | | 1 | EW6 1991 | CW | | 1.22 | 0.18 | 131 | 2 | 85 | 98.5 | |
| 2000 | Bachand and Horne | 1 | all cells in a series | CW | FWS | 6.44 | 6.12 | | | 5 | | 2 dates but only one here ➔ But very high load removal according to the authors (no data) |
| | | 1 | cell 1 | CW | FWS | 6.44 | 6.41 | | | 0.47 | | |
| 2000 | Spieles and Mitsch | 1 | 1 wastewater treatment CW | CW | FWS | 12.5 | 7.7 | 12.3 | 7.4 | 38 | 29.3 | Inlet concentration in $NO_3$+$NO_2$-N; Inlet load in kg$NO_3$+$NO_2$-N/ha/d; Conc removal calculated but load removal comes from from paper |
| | | 1 | ORW1 | CW | FWS | 4.6 | 3.4 | 4.6 | 3 | 26 | 39.8 | |
| | | 1 | ORW2 | CW | FWS | 4.6 | 3.6 | 4.7 | 2.9 | 22 | 36.7 | |
| 2009 | Kadlec | 1 | 1 outlet of E8 | Nat | FWS | 1.62 | 0.16 | | | 90 | | Average over a 30-yr period; conc removal calculated |
| | | 1 | 1 outlet of E8 | Nat | FWS | 1.14 | 0.14 | | | 88 | | |
| | | 1 | 1 outlet of E9 | Nat | FWS | 1.62 | 0.203 | | | 87 | | |
| | | 1 | 1 outlet of E9 | Nat | FWS | 1.14 | 0.146 | | | 87 | | |
| 2002 | Braskerud | 1 | A | CW | | 2.28 | 2.29 | | | 0 | | Increase in nitrate retention when decrease in hydraulic load; summer=high denitrification; fall=low denitrification due to large |
| | | 1 | C | CW | | 2.16 | 2.18 | | | -1 | | |
| | | 1 | F | CW | | 0.75 | 0.73 | | | 3 | | |
| | | 1 | G1 | CW | | 2.77 | 2.5 | | | 9 | | |

| Year | Authors | Number of wetland system | Wetland identification | Type Nat vs. CW | Flow regime FWS vs. SSF | Conc in mg/L | Conc out mg/L | Load in mgN/ m²/yr | Load out mgN/ m²/yr | Nitrate removal conc % | Nitrate removal load % | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | G2 | CW | | 2.77 | 2.57 | | | 7 | | flood event that washed off carbon |
| 2004 | Fink and Mitsch | 1 | total 4 cells in a series | CW | | 0.796 | 0.476 | | | 40.2 | | |
| 2004 | Mbulingue | 1 | Control | CW | SSF | 2.48 | 1.71 | | | 29.7 | 42 | |
| | | 1 | Typha | CW | SSF | 2.48 | 1.38 | | | 43.8 | 60.4 | |
| | | 1 | Colocasia | CW | SSF | 2.48 | 151 | | | 39.2 | 55.5 | |
| 2005 | Mitsch et al. | 1 | Wetland 1 1994 | CW | FWS | | | 57.2 | 41.6 | 49 | 27 | |
| | | 1 | Wetland 2 1994 | CW | FWS | | | 57.9 | 45.2 | 46 | 22 | |
| | | 1 | Wetland 1 1995 | CW | FWS | | | 85.8 | 67.9 | 36 | 21 | |
| | | 1 | Wetland 2 1995 | CW | FWS | | | 85.8 | 59.9 | 42 | 30 | |
| | | 1 | Wetland 1 1996 | CW | FWS | | | 58.4 | 39.1 | 33 | 33 | |
| | | 1 | Wetland 2 1996 | CW | FWS | | | 58.5 | 43.5 | 25 | 26 | |
| | | 1 | Wetland 1 1997 | CW | FWS | | | 211 | 130 | 17 | 38 | |
| | | 1 | Wetland 2 1997 | CW | FWS | | | 215 | 124 | 18 | 42 | |
| | | 1 | Wetland 1 1998 | CW | FWS | | | 136 | 95 | 33 | 30 | |
| | | 1 | Wetland 2 1998 | CW | FWS | | | 138 | 83 | 39 | 40 | |
| | | 1 | Wetland 1 1999 | CW | FWS | | | 78.6 | 57.3 | 30 | 27 | |
| | | 1 | Wetland 2 1999 | CW | FWS | | | 81.9 | 51 | 33 | 38 | |
| | | 1 | Wetland 1 2000 | CW | FWS | | | 129.3 | 81.2 | 34 | 37 | |
| | | 1 | Wetland 2 2000 | CW | FWS | | | 128.4 | 80 | 44 | 38 | |
| | | 1 | Wetland 1 2001 | CW | FWS | | | 112.2 | 63.1 | 35 | 44 | |
| | | 1 | Wetland 2 2001 | CW | FWS | | | 106.2 | 68.9 | 23 | 35 | |
| | | 1 | Wetland 1 2003 | CW | FWS | | | 104.9 | 62.3 | 41 | 41 | |
| | | 1 | Wetland 2 2003 | CW | FWS | | | 98.7 | 47.5 | 44 | 52 | |
| | | 1 | caernavon div 1992 | Nat | | | | 5.6 | 0.17 | 97 | 97 | |
| | | 1 | caernavon div 1993 | Nat | | | | 73 | 1.54 | 79 | 79 | |
| | | 1 | caernavon div 1994 | Nat | | | | 12.7 | 4.2 | 67 | 67 | |
| | | 1 | caernavon div 2001 10 mk² | Nat | | | | 50 | 19 | 62 | 62 | |
| | | 1 | caernavon div 2001 30 mk² | Nat | | | | 84 | 38 | 55 | 55 | |
| | | 1 | caernavon div 2001 50 mk² | Nat | | | | 251 | 161 | 36 | 36 | |

| Year | Authors | Number of wetland system | Wetland identification | Type Nat vs. CW | Flow regime FWS vs. SSF | Conc in mg/L | Conc out mg/L | Load in mgN/ m²/yr | Load out mgN/ m²/yr | Nitrate removal conc % | Nitrate removal load % | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2006 | Kovacic | 1 | W1 1998 | CW | | | | 38.6 | 25.9 | NA | 40 | Only tile-drainage inlet given |
| | | 1 | W2 1998 | CW | | | | 291.5 | 233.8 | NA | 31 | |
| | | 1 | W1 1999 | CW | | | | 60.9 | 54.5 | NA | 16 | |
| | | 1 | W2 1999 | CW | | | | 355.1 | 214.8 | NA | 43 | |
| | | 1 | W1 study period | CW | | | | | | 42 | | |
| | | 1 | W2 study period | CW | | | | | | 31 | | |
| 2006 | Nahlik and Mitsch | 1 | Lecheria 1-2 | CW | | | | | | -207 | -207 | |
| | | 1 | Lecheria 3-4 | CW | | | | | | -77 | -77 | |
| | | 1 | LaPA | CW | | | | | | 89 | 89 | |
| | | 1 | Plata de papel | CW | | | | | | 84 | 84 | |
| | | 1 | Relleno sanitario | CW | | | | | | -78 | -78 | |
| 2009 | Hathaway and Hunt | 1 | Wetland 1 | CW | | 0.44 | 0.14 | | | 67 | | |
| | | 1 | Wetland 2 | CW | | 0.14 | 0.08 | | | 47 | | |
| | | 1 | Wetland 3 | CW | | 0.08 | 0.07 | | | 12 | | |
| 2008 | Knox | 1 | Non-degraded ref wetland | Nat | | 0.2 | | | | 60 | | |
| | | 1 | Channelized wetland | Nat | | 0.31 | | | | -25 | | |
| 2000 | Reilly et al. | 1 | Total system phase 1 | CW | | | | | | 100 | | |
| | | 1 | Total system phase 2 | CW | | | | | | 84 | | |
| | | 1 | Total system phase 3 | CW | | | | | | 45 | | |
| 2005 | Tanner et al. | 1 | Year 1 | CW | | 10.6 | 6.5 | | | 39 | 44 | Concentrations read on figure and efficiencies for concentration reductions were calculated |
| | | 1 | Year 2 | CW | | 11 | 9.5 | | | 14 | 16 | |

**Table A 2: Wetland and nitrate literature summary**

**Appendix III Pesticide application records at Bray from 2002 to 2010**

| Type | Product | Date | Application dose | unit of application dose | Plot | Active ingredient (A.I.) | Concentration of A.I | unit of A.I. | Area of application (ha) | Applied dose (g/ha) | Total applied mass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbicide | Glyfoflash | 21/08/2009 | 1.8 | l/ha | P1 | glyphosate | 360 | g/L | 14 | 648 | 9176 |
| | u46D | 02/09/2009 | 1 | l/ha | P1 | 2,4-D | 480 | g/L | 14 | 480 | 6797 |
| | defi | 12/11/2009 | 2 | l/ha | P1 | prosulfocarbe | 800 | g/L | 14 | 1600 | 22400 |
| | Tablo 700 | 12/11/2009 | 2.6 | l/ha | P1 | chlorotoluron | 700 | g/L | 14 | 1820 | 25480 |
| | Octogon | 23/03/2010 | 280 | g/ha | P1 | florasulam | 22.8 | g/kg | 14 | 6 | 90 |
| | Octogon | 23/03/2010 | 280 | g/ha | P1 | cloquintocet mexyl | 68.3 | g/kg | 14 | 19 | 271 |
| | Octogon | 23/03/2010 | 280 | g/ha | P1 | pyroxsulame | 68.3 | g/kg | 14 | 19 | 271 |
| | Archipel | 05/04/2010 | 150 | g/ha | P1 | mesosulfuron methyl | 30 | g/kg | 10 | 5 | 45 |
| | Archipel | 05/04/2010 | 150 | g/ha | P1 | iodosulfuron-methyl | 30 | g/kg | 10 | 5 | 45 |
| | Buggy plus | 14/08/2009 | 2 | l/ha | P5 | glyphosate | 270 | g/L | 16 | 540 | 8662 |
| | colzor trio | 25/08/2009 | 3.5 | l/ha | P5 | dimethachlor | 187.5 | g/L | 16 | 656 | 10526 |
| | colzor trio | 25/08/2009 | 3.5 | l/ha | P5 | clomazone | 30 | g/L | 16 | 105 | 1684 |
| | colzor trio | 25/08/2009 | 3.5 | l/ha | P5 | napopramide | 187.5 | g/L | 16 | 656 | 10526 |
| | cent 7 | 12/11/2009 | 0.5 | l/ha | P5 | isoxaben | 125 | g/L | 13 | 63 | 813 |
| | stratos ultra+dash | 23/03/2010 | 1.5 | l/ha | P5 | cycloxydime | 100 | g/L | 9 | 150 | 1275 |
| | Colzor trio | 25/08/2009 | 3.5 | l/ha | P4 | dimethachlor | 187.5 | g/L | 7 | 656 | 4463 |
| | colzor trio | 25/08/2009 | 3.5 | l/ha | P4 | clomazone | 30 | g/L | 7 | 105 | 714 |
| | colzor trio | 25/08/2009 | 3.5 | l/ha | P4 | napopramide | 187.5 | g/L | 7 | 656 | 4463 |
| | Callisto | 20/11/2009 | 0.15 | l/ha | P4 | mesotrione | 100 | g/L | 7 | 15 | 102 |
| | stratos ultra+dash | 23/03/2010 | 1.5 | l/ha | P4 | cycloxydime | 100 | g/L | 3 | 150 | 495 |
| | Glyfoflash | 15/09/2009 | 3 | l/ha | P3 | glyphosate | 360 | g/L | 3 | 1080 | 3240 |
| | u46D | 15/09/2009 | 1.4 | l/ha | P3 | 2,4-D | 480 | g/L | 3 | 672 | 2016 |
| | puzzle | 10/11/2009 | 2.5 | l/ha | P3 | bifenox | 150 | g/L | 3 | 375 | 1125 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | puzzle | 10/11/2009 | 2.5 | l/ha | P3 | diflufenican | 26.7 | g/L | 3 | 67 | 200 |
| | puzzle | 10/11/2009 | 2.5 | l/ha | P3 | isoproturon | 333.4 | g/L | 3 | 834 | 2501 |
| | Archipel | 05/04/2010 | 0.15 | kg/ha | P3 | mesosulfuron methyl | 30 | g/kg | 3 | 5 | 14 |
| | Archipel | 05/04/2010 | 0.15 | kg/ha | P3 | iodosulfuron-methyl | 30 | g/kg | 3 | 5 | 14 |
| | Fluo 250 CS | ? | 2.5 | l/ha | P2 | flurochloridone | 250 | g/L | ? | 625 | |
| Fungicide | Fandango S | 20/04/2010 | 0.9 | l/ha | P1 | prothioconazole | 100 | g/L | 14 | 90 | 1274 |
| | Fandango S | 20/04/2010 | 0.9 | l/ha | P1 | fluoxastrobine | 50 | g/L | 14 | 45 | 637 |
| | Altitude | 08/05/2010 | 0.5 | l/ha | P1 | kresoxim-methyl | 125 | g/L | 14 | 63 | 885 |
| | Altitude | 08/05/2010 | 0.5 | l/ha | P1 | epoxiconazole | 125 | g/L | 14 | 63 | 885 |
| | Altitude | 08/05/2010 | 0.5 | l/ha | P1 | fenpropimorphe | 150 | g/L | 14 | 75 | 1062 |
| | Pictor pro | 19/04/2010 | 0.4 | kg/ha | P5 | boscalid | 50 | % | 16 | 200 | 3208 |
| | Pictor pro | 22/04/2010 | 0.25 | kg/ha | P4 | boscalid | 50 | % | 7 | 125 | 850 |
| | Sunnorg Pro | 22/04/2010 | 0.4 | l/ha | P4 | metconazole | 90 | g/L | 7 | 36 | 245 |
| | Altitude | 08/05/2010 | 0.5 | l/ha | P3 | kresoxim-methyl | 125 | g/L | 3 | 63 | 188 |
| | Altitude | 08/05/2010 | 0.5 | l/ha | P3 | epoxiconazole | 125 | g/L | 3 | 63 | 188 |
| | Altitude | 08/05/2010 | 0.5 | l/ha | P3 | fenpropimorphe | 150 | g/L | 3 | 75 | 225 |
| Insecticide | Karate K | 15/09/2009 | 1 | l/ha | P5 | lambda-cyhalothrin | 5 | g/L | 16 | 5 | 80 |
| | Karate K | 15/09/2009 | 1 | l/ha | P5 | pirimicarb | 100 | g/L | 16 | 100 | 1604 |
| | Karate Zeon | 23/03/2010 | 0.05 | l/ha | P5 | lambda-cyhalothrin | 100 | g/L | 16 | 5 | 80 |
| | Karate Zeon | 23/03/2010 | 0.05 | l/ha | P4 | lambda-cyhalothrin | 100 | g/L | 7 | 5 | 34 |

**Table A 3: 2009-2010 pesticides application**

| Type | Product | Date | Application dose | unit of application dose | Plot | Active ingredient (A.I.) | Concentration of A.I | unit of A.I. | Area of application (ha) | Applied dose (g/ha) | Total applied mass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbicide | Flurasan 480 | 25/08/2008 | 1940 | g/ha | P1 | trifluraline | 480 | g/L | 14 | 931 | 13186 |
| | Colzor Trio | 28/08/2008 | 2.64 | l/ha | P1 | dimethachlor | 187.5 | g/L | 14 | 495 | 7009 |
| | Colzor Trio | 28/08/2008 | 2.64 | l/ha | P1 | clomazone | 30 | g/L | 14 | 79 | 1121 |
| | Colzor Trio | 28/08/2008 | 2.64 | l/ha | P1 | napopramide | 187.5 | g/L | 14 | 495 | 7009 |
| | KERB FLO | 09/12/2008 | 1.5 | l/ha | P1 | propyzamide | 400 | g/L | 5 | 600 | 2820 |
| | Glyphogan | 05/09/2008 | 2500 | g/ha | P5 | glyphosate | 360 | g/L | 9 | 900 | 7650 |
| | PROTUGAN | 10/12/2008 | 2.25 | l/ha | P5 | isoproturon | 500 | g/L | 15 | 1125 | 16875 |
| | Matin EL | 10/12/2008 | 2.23 | l/ha | P5 | isoproturon | 500 | g/L | 1 | 1115 | 1115 |
| | BAGHERA | 12/03/2009 | 1.45 | l/ha | P5 | fenoxaprop-p-ethyl | 20 | g/L | 16 | 29 | 465 |
| | BAGHERA | 12/03/2009 | 1.45 | l/ha | P5 | diclofop-methyl | 250 | g/L | 16 | 363 | 5815 |
| | BAGHERA | 12/03/2009 | 1.45 | l/ha | P5 | mefenpyr-diethyl | 40 | g/L | 16 | 58 | 930 |
| | HARMONY EXTRA | 17/04/2009 | 40 | g/ha | P5 | tribenuron-methyle | 25 | % | 16 | 10 | 160 |
| | HARMONY EXTRA | 17/04/2009 | 40 | g/ha | P5 | thifensulfuron-methyle | 50 | % | 16 | 20 | 321 |
| | GRIVOLAX | 03/04/2009 | 2 | l/ha | P3 | glyphosate | 360 | g/L | 3 | 720 | 2160 |
| | Racer ME | 27/04/2009 | 2.5 | l/ha | P3 | flurochloridone | 250 | g/L | 3 | 625 | 1875 |
| | Glyphogan | 14/08/2009 | 2700 | g/ha | P4 | glyphosate | 360 | g/L | 7 | 972 | 6610 |
| | nicanor | 14/08/2009 | 6.5 | g/ha | P4 | metsulfuron methyle | 200 | g/kg | 7 | 1 | 9 |
| | PROTUGAN | 23/12/2008 | 2.2 | l/ha | P4 | isoproturon | 500 | g/L | 7 | 1100 | 7480 |
| | PROTUGAN | 22/12/2008 | 2.2 | l/ha | P5 | isoproturon | 500 | g/L | 8 | 1100 | 8800 |
| | ARCHIPEL | 06/03/2009 | 125 | g/ha | P4 | mesosulfuron methyl | 30 | g/kg | 7 | 4 | 26 |
| | ARCHIPEL | 06/03/2009 | 125 | g/ha | P4 | iodosulfuron-methyl | 30 | g/kg | 7 | 4 | 26 |
| | Cadeli | 02/06/2009 | 1 | l/ha | P2 | bromoxynil | 225 | g/L | 1 | 225 | 236 |
| | Trophée | 27/04/2009 | 4.57 | l/ha | P2 | acetochlore | 400 | g/L | 0.4 | 1828 | 640 |
| | Trophée | 27/04/2009 | 4.57 | l/ha | P2 | dichlormide | 66.7 | g/L | 0.4 | 305 | 107 |
| | Lagon | 27/04/2009 | 0.57 | l/ha | P2 | isoxaflutole | 75 | g/L | 0.4 | 43 | 15 |
| | Lagon | 27/04/2009 | 0.57 | l/ha | P2 | aclonifen | 500 | g/L | 0.4 | 285 | 100 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cadeli | 02/06/2009 | 1 | l/ha | P2 | bromoxynil | 225 | g/L | 1 | 225 | 152 |
| | Racer ME | 27/04/2009 | 2.5 | l/ha | P2 | flurochloridone | 250 | g/L | 2 | 625 | 1263 |
| | Trophée | 27/04/2009 | 4.57 | l/ha | P2 | acetochlore | 400 | g/L | 1 | 1828 | 1773 |
| | Trophée | 27/04/2009 | 4.57 | l/ha | P2 | dichlormide | 66.7 | g/L | 1 | 305 | 296 |
| | Lagon | 27/04/2009 | 0.57 | l/ha | P2 | isoxaflutole | 75 | g/L | 1 | 43 | 41 |
| | Lagon | 27/04/2009 | 0.57 | l/ha | P2 | aclonifen | 500 | g/L | 1 | 285 | 276 |
| Fungicide | Pictor Pro | 23/04/2009 | 410 | g/ha | P1 | boscalid | 50 | % | 14 | 205 | 2903 |
| | FANDANGO | 14/04/2009 | 0.8 | l/ha | P5 | prothioconazole | 100 | g/L | 16 | 80 | 1283 |
| | FANDANGO | 14/04/2009 | 0.8 | l/ha | P5 | fluoxastrobine | 50 | g/L | 16 | 40 | 642 |
| | FANDANGO | 02/05/2009 | 1 | l/ha | P5 | prothioconazole | 100 | g/L | 14 | 100 | 1448 |
| | FANDANGO | 02/05/2009 | 1 | l/ha | P5 | fluoxastrobine | 50 | g/L | 14 | 50 | 724 |
| | Altitud | 13/05/2009 | 0.55 | l/ha | P4 | kresoxim-methyl | 125 | g/L | 6 | 69 | 421 |
| | Altitud | 13/05/2009 | 0.55 | l/ha | P4 | fenpropimorphe | 150 | g/L | 6 | 83 | 506 |
| | Altitud | 13/05/2009 | 0.55 | l/ha | P4 | epoxiconazole | 125 | g/L | 6 | 69 | 421 |
| Insecticide | star 100 | 15/09/2008 | 0.075 | l/ha | P1 | alphamethrin | 100 | g/L | 14 | 8 | 106 |
| | KARATE ZEON | 11/03/2009 | 0.075 | l/ha | P1 | lambda-cyhalothrin | 100 | g/L | 14 | 8 | 106 |
| | KARATE ZEON | 18/04/2009 | 0.05 | l/ha | P1 | lambda-cyhalothrin | 100 | g/L | 14 | 5 | 71 |

**Table A 4: 2008-2009 pesticides applications**

| Type | Product | Date | Application dose | unit of application dose | Plot | Active ingredient (A.I.) | Concentration of A.I | unit of A.I. | Area of application (ha) | Applied dose (g/ha) | Total applied mass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbicide | BUGGY | 10/08/2007 | 2 | l/ha | P1 | Glyphosate | 450 | g/L | 14 | 900 | 12744 |
| | Tartan | 17/08/2007 | 2.3 | l/ha | P5 | Glyphosate | 360 | g/L | 16 | 828 | 13281 |
| | Novall | 03/09/2007 | 2.11 | l/ha | P4 | Quinmerac | 100 | g/L | 4 | 211 | 859 |
| | Novall | 03/09/2007 | 2.11 | l/ha | P4 | Metazachlor | 400 | g/L | 4 | 844 | 3435 |
| | Stratos + Dash | 13/09/2007 | 0.71 | l/ha | P4 | cycloxydime | 100 | g/L | 4 | 710 | 2890 |
| | Tartan | 18/10/2007 | 1.5 | l/ha | P5 | Glyphosate | 360 | g/L | 16 | 540 | 8662 |
| | First | 02/11/2007 | 1.1 | l/ha | P1 | diflufenican | 40 | g/L | 14 | 44 | 623 |
| | First | 02/11/2007 | 1.1 | l/ha | P1 | ionoxynil | 75 | g/L | 14 | 83 | 1168 |
| | First | 02/11/2007 | 1.1 | l/ha | P1 | bromoxynil | 125 | g/L | 14 | 138 | 1947 |
| | Protugan | 02/11/2007 | 2.4 | l/ha | P1 | isoproturon | 500 | g/L | 14 | 1200 | 16992 |
| | cent 7 | 13/11/2007 | 0.3 | l/ha | P4 | isoxaben | 125 | g/L | 4 | 38 | 153 |
| | chrono | 15/11/2007 | 0.6 | kg/ha | P4 | pyridate | 36 | % | 4 | 22 | 88 |
| | chrono | 15/11/2007 | 0.6 | kg/ha | P4 | piclorame | 1.12 | % | 4 | 1 | 3 |
| | Zeus | 21/02/2008 | 1.8 | l/ha | P1 | fenoxaprop-p-ethyl | 20 | g/L | 14 | 36 | 510 |
| | Zeus | 21/02/2008 | 1.8 | l/ha | P1 | mefenpyr-diethyl | 40 | g/L | 14 | 72 | 1020 |
| | Zeus | 21/02/2008 | 1.8 | l/ha | P1 | Diclofop methyl | 250 | g/L | 14 | 450 | 6372 |
| | Archipel | 22/02/2008 | 150 | g/ha | P5 | Iodosulfuron | 30 | g/kg | 16 | 5 | 72 |
| | Archipel | 22/02/2008 | 150 | g/ha | P5 | Mesosulfuron | 30 | g/kg | 16 | 5 | 72 |
| | Archipel | 25/02/2008 | 180 | g/ha | P3 | iodosulfuron-methyl | 30 | g/kg | 3 | 5 | 16 |
| | Archipel | 25/02/2008 | 180 | g/ha | P3 | Mesosulfuron | 30 | g/kg | 3 | 5 | 16 |
| | Stratos + Dash | 14/03/2008 | 1.1 | l/ha | P4 | cycloxydime | 100 | g/L | 4 | 110 | 448 |
| | Flurasan 480 | 11/05/2008 | 2 | l/ha | P2 | trifluraline | 480 | g/L | 7 | 960 | 6240 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pampa | 05/06/2008 | 1 | l/ha | P2 | nicosulfuron | 40 | g/L | 2 | 40 | 90 |
| | Chardex | 10/06/2008 | 1 | l/ha | P5 | clopyralid | 35 | g/L | 5 | 35 | 158 |
| | Chardex | 10/06/2008 | 1 | l/ha | P5 | 2,4-MCPA | 350 | g/L | 5 | 350 | 1575 |
| | Chardex | 10/06/2008 | 1 | l/ha | P3 | clopyralid | 35 | g/L | 3 | 35 | 105 |
| | Chardex | 10/06/2008 | 1 | l/ha | P3 | 2,4-MCPA | 350 | g/L | 3 | 350 | 1050 |
| | Mikado | 20/06/2008 | 0.5 | l/ha | P2 | sulcotrione | 300 | g/L | 7 | 150 | 1049 |
| | Stratos + Dash | ? | 1.4 | l/ha | P4 | cycloxydime | 100 | g/L | 4 | 140 | 570 |
| Fungicide | fandango | 04/04/2008 | 1 | l/ha | P1 | prothioconazole | 100 | g/L | 14 | 100 | 1416 |
| | fandango | 04/04/2008 | 1 | l/ha | P1 | fluoxastrobine | 50 | g/L | 14 | 50 | 708 |
| | Altitude | 15/04/2008 | 0.43 | l/ha | P5 | kresoxim-methyl | 125 | g/L | 16 | 54 | 862 |
| | Altitude | 15/04/2008 | 0.43 | l/ha | P5 | epoxiconazole | 125 | g/L | 16 | 54 | 862 |
| | Altitude | 15/04/2008 | 0.43 | l/ha | P5 | fenpropimorphe | 150 | g/L | 16 | 65 | 1035 |
| | Altitude | 15/04/2008 | 0.43 | l/ha | P3 | kresoxim-methyl | 125 | g/L | 3 | 54 | 161 |
| | Altitude | 15/04/2008 | 0.43 | l/ha | P3 | epoxiconazole | 125 | g/L | 3 | 54 | 161 |
| | Altitude | 15/04/2008 | 0.43 | l/ha | P3 | fenpropimorphe | 150 | g/L | 3 | 65 | 194 |
| | Pictor pro | 16/04/2008 | 0.32 | l/ha | P4 | boscalid | 500 | g/kg | 7 | x | |
| | fandango | 27/04/2008 | 0.9 | l/ha | P1 | fluoxastrobine | 50 | g/L | 14 | 45 | 637 |
| | fandango | 27/04/2008 | 0.9 | l/ha | P1 | prothioconazole | 100 | g/L | 14 | 90 | 1274 |
| | fandango | 07/05/2008 | 0.92 | l/ha | P5 | fluoxastrobine | 50 | g/L | 16 | 46 | 738 |
| | fandango | 07/05/2008 | 0.92 | l/ha | P5 | prothioconazole | 100 | g/L | 16 | 92 | 1476 |
| | fandango | 07/05/2008 | 0.92 | l/ha | P3 | fluoxastrobine | 50 | g/L | 3 | 46 | 138 |
| | fandango | 07/05/2008 | 0.92 | l/ha | P3 | prothioconazole | 100 | g/L | 3 | 92 | 276 |
| | Opus | 14/05/2008 | 0.5 | l/ha | P3 | epoxiconazole | 125 | g/L | 3 | 63 | 188 |
| | Sunorg Pro | 20/05/2008 | 0.36 | l/ha | P4 | metconazole | 90 | g/L | 7 | 32 | 221 |
| Insecticide | Cytrine L | 14/03/2008 | 0.25 | l/ha | P4 | cypermethrin | 100 | g/L | 7 | 25 | 171 |
| | Cytrine L | 16/04/2008 | 0.25 | l/ha | P4 | cypermethrin | 100 | g/L | 7 | 25 | 171 |

**Table A 5: 2007-2008 pesticides applications**

| Type | Product | Date | Application dose | unit of application dose | Plot | Active ingredient (A.I.) | Concentration of A.I | unit of A.I. | Area of application (ha) | Applied dose (g/ha) | Total applied mass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbicide | Tréflan | 28/08/06 | | | P5 | trifluraline | | | 16 | 960 | 15398 |
| | Dévrinol | 28/08/06 | | | P5 | Napropamide | | | 16 | 900 | 14436 |
| | Kerb Flo | 01/12/06 | | | P5 | Propyzamide | | | 16 | 135 | 2160 |
| | Puma Ls | 12/11/06 | | | P1 | fenoxaprop-p-ethyl | | | 14 | 41 | 586 |
| | Puma Ls | 12/11/06 | | | P1 | mefenpyr-diethyl | | | 14 | 11 | 159 |
| | First | 12/11/06 | | | P1 | diflufenican | | | 14 | 20 | 283 |
| | First | 12/11/06 | | | P1 | ioxynil | | | 14 | 38 | 531 |
| | First | 12/11/06 | | | P1 | bromoxynil | | | 14 | 63 | 885 |
| | Archipel | 17/03/07 | | | P1 | mesosulfuron methyl | | | 14 | 5 | 64 |
| | Archipel | 17/03/07 | | | P1 | iodosulfuron-methyl | | | 14 | 5 | 64 |
| | Puma Ls | 12/11/06 | | | P4 | fenoxaprop-p-ethyl | | | 9 | 41 | 359 |
| | Puma Ls | 12/11/06 | | | P4 | mefenpyr-diethyl | | | 9 | 11 | 97 |
| | First | 12/11/06 | | | P4 | diflufenican | | | 9 | 20 | 173 |
| | First | 12/11/06 | | | P4 | ioxynil | | | 9 | 38 | 325 |
| | First | 12/11/06 | | | P4 | bromoxynil | | | 9 | 63 | 542 |
| | Archipel | 17/03/07 | | | P4 | mesosulfuron methyl | | | 9 | 5 | 39 |
| | Archipel | 17/03/07 | | | P4 | iodosulfuron-methyl | | | 9 | 5 | 39 |
| | Archipel | 17/03/07 | | | P2 | mesosulfuron methyl | | | 9 | 5 | 41 |
| | Archipel | 17/03/07 | | | P2 | iodosulfuron-methyl | | | 9 | 5 | 41 |
| | Archipel | 17/03/07 | | | P2 | mesosulfuron methyl | | | 9 | 5 | 41 |
| | Archipel | 17/03/07 | | | P2 | iodosulfuron-methyl | | | 9 | 5 | 41 |
| | Basamaïs | ? | | | P5 | Bentazone | | | 7 | 960 | 6451 |
| Fungicide | Kimono | ?? | | | P5 | procymidone | | | 16 | 5 | 80 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Opus | ? | P1 | epoxiconazole | 14 | 100 | 1416 |
| | Opus | ? | P4 | epoxiconazole | 9 | 100 | 867 |
| | Opus | ? | P2 | epoxiconazole | 9 | 100 | 909 |
| Insecticide | Karaté Zéon | 02/11/06 | P5 | lambda-cyhalothrin | 16 | 5 | 80 |
| | Karaté Zéon | 12/04/07 | P5 | lambda-cyhalothrin | 16 | 500 | 8020 |
| | Karaté Zéon | 02/11/06 | P5 | lambda-cyhalothrin | 16 | 5 | 80 |
| | Cyplan | ? | P1 | Cypermethrine | 14 | 20 | 283 |
| | Cyplan | ? | P4 | Cypermethrine | 9 | 20 | 173 |

**Table A 6: 2006-2007 pesticides applications**

| Type | Product | Date | Application dose | unit of application dose | Plot | Active ingredient (A.I.) | Concentration of A.I | unit of A.I. | Area of application (ha) | Applied dose (g/ha) | Total applied mass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbicide | Chlortoluron | 05/11/2005 | | | P5 | Chlorotoluron | | | 16 | 1500 | 24060 |
| | Baghera | 05/11/2005 | | | P5 | fenoxaprop-p-ethyl | | | 16 | 20 | 321 |
| | kino | 19/04/2006 | | | P5 | MCPP | | | 16 | 260 | 4170 |
| | Baghera | 05/11/2005 | | | P5 | diclofop-methyl | | | 16 | 250 | 4010 |
| | kino | 19/04/2006 | | | P5 | DCPP | | | 16 | 620 | 9945 |
| | Baghera | 05/11/2005 | | | P5 | mefenpyr-diethyl | | | 16 | 40 | 642 |
| | kino | 19/04/2006 | | | P5 | 2,4 MCPA | | | 16 | 320 | 5133 |
| | colzor trio | 02/09/2005 | | | P1 | napropamide | | | 10 | 929 | 9293 |
| | colzor trio | 02/09/2005 | | | P1 | clomazone | | | 10 | 149 | 1487 |
| | colzor trio | 02/09/2005 | | | P1 | dimethachlor | | | 10 | 929 | 9293 |
| | stratos | 20/10/2005 | | | P1 | cycloxydime | | | 10 | 170 | 1699 |
| | Tréflan | ? | | | P4 | trifluraline | | | 9 | 960 | 8323 |
| | Dévrinol | ? | | | P4 | napropamide | | | 9 | 900 | 7803 |
| | Basamaïs | ? | | | P2 | bentazone | | | 9 | 1200 | 10908 |
| | Archipel | ? | | | P3 | mesosulfuron methyl | | | 7 | 5 | 30 |
| | Archipel | ? | | | P3 | iodosulfuron-methyl | | | 7 | 5 | 30 |
| | allie | ? | | | P3 | metsulfuron methyl | | | 7 | 6 | 40 |
| Fungicide | sphère | n/a | | | P5 | cyproconazole | | | 16 | 75 | 1203 |
| | citadelle | n/a | | | P5 | cyproconazole | | | 16 | 263 | 4211 |
| | sphère | n/a | | | P5 | trifloxystrobine | | | 16 | 32 | 513 |
| | citadelle | n/a | | | P5 | chlorothalonil | | | 16 | 28 | 449 |
| | Kimono | 10/05/2006 | | | P1 | procymidone | | | 10 | 708 | 7080 |
| | Kimono | ? | | | P4 | procymidone | | | 9 | 500 | 4335 |
| | sphère | ? | | | P3 | cyproconazole | | | 7 | 94 | 630 |
| | sphère | ? | | | P3 | trifloxystrobine | | | 7 | 40 | 269 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | opus | ? | P3 | epoxiconazole | 7 | 63 | 420 |
| Insecticide | Karaté Zéon | 10/03/2006 | P1 | lambda-cyhalothrin | 10 | 7 | 71 |
| | Karaté Zéon | ? | P4 | lambda-cyhalothrin | 9 | 5 | 43 |

**Table A 7: 2005-2006 pesticides applications**

| Type | Product | Date | Application dose | unit of application dose | Plot | Active ingredient (A.I.) | Concentration of A.I | unit of A.I. | Area of application (ha) | Applied dose (g/ha) | Total applied mass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbicide | Puma LS | 18/10/2004 | | | P5 | fenoxaprop-p-ethyl | | | 16 | 35 | 553 |
| | Puma LS | 18/10/2004 | | | P5 | mefenpyr-diethyl | | | 16 | 9 | 150 |
| | First | 18/10/2004 | | | P5 | diflufenicanil | | | 16 | 24 | 385 |
| | First | 18/10/2004 | | | P5 | ioxynil | | | 16 | 45 | 722 |
| | First | 18/10/2004 | | | P5 | bromoxynil | | | 16 | 75 | 1203 |
| | Archipel | 08/04/2005 | | | P5 | mesosulfuron methyl | | | 16 | 4 | 60 |
| | Archipel | 08/04/2005 | | | P5 | iodosulfuron-methyl | | | 16 | 4 | 60 |
| | Quartz | 29/10/2004 | | | P1 | diflufenicanil | | | 10 | 177 | 1770 |
| | Quartz | 29/10/2004 | | | P1 | isoproturon | | | 10 | 1416 | 14160 |
| | Illoxan CE | 29/10/2004 | | | P1 | diclofop-methyl | | | 10 | 268 | 2676 |
| | Baghera | 26/11/2004 | | | P1 | fenoxaprop-p-ethyl | | | 10 | 57 | 566 |
| | Baghera | 26/11/2004 | | | P1 | diclofop-methyl | | | 10 | 708 | 7080 |
| | Baghera | 26/11/2004 | | | P1 | mefenpyr-diethyl | | | 10 | 113 | 1133 |
| | Baghera | 21/03/2005 | | | P1 | fenoxaprop-p-ethyl | | | 10 | 12 | 120 |
| | Baghera | 21/03/2005 | | | P1 | diclofop-methyl | | | 10 | 150 | 1500 |
| | Baghera | 21/03/2005 | | | P1 | mefenpyr-diethyl | | | 10 | 24 | 240 |
| | Quartz | 29/10/2004 | | | P4 | diflufenicanil | | | 9 | 125 | 1084 |
| | Quartz | 29/10/2004 | | | P4 | isoproturon | | | 9 | 1000 | 8670 |
| | Illoxan CE | 29/10/2004 | | | P4 | diclofop-methyl | | | 9 | 265 | 2294 |
| | Attribut | 12/04/2005 | | | P4 | propoxycarbazone sodium | | | 9 | 10 | 84 |
| | Archipel | 21/03/2005 | | | P2 | mesosulfuron methyl | | | 9 | 8 | 68 |
| | Archipel | 21/03/2005 | | | P2 | iodosulfuron-methyl | | | 9 | 8 | 68 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | tréflan | 09/05/2005 | P3 | trifluraline | 7 | 960 | 6451 |
| | novall | 12/05/2005 | P3 | quinmérac | 7 | 150 | 1008 |
| | novall | 12/05/2005 | P3 | metazachlor | 7 | 600 | 4032 |
| | racer | 12/05/2005 | P3 | flurochloridone | 7 | 250 | 1680 |
| Fungicide | sphère | 15/04/2005 | P5 | cyproconazole | 16 | 94 | 1504 |
| | sphère | 15/04/2005 | P5 | trifloxystrobine | 16 | 40 | 642 |
| | altitude | 06/05/2005 | P5 | epoxiconazole | 16 | 63 | 1003 |
| | altitude | 06/05/2005 | P5 | kresoxim-methyl | 16 | 63 | 1003 |
| | altitude | 06/05/2005 | P5 | fenpropimorphe | 16 | 75 | 1203 |
| | Amistar | 01/04/2005 | P1 | azoxystrobine | 10 | 177 | 1770 |
| | Unix | 01/04/2005 | P1 | cyprodinil | 10 | 531 | 5310 |
| | Amistar | 01/05/2005 | P1 | azoxystrobine | 10 | 177 | 1770 |
| | sphère | 14/04/2005 | P4 | cyproconazole | 9 | 94 | 813 |
| | sphère | 14/04/2005 | P4 | trifloxystrobine | 9 | 40 | 347 |
| | altitude | 06/05/2005 | P4 | epoxiconazole | 9 | 63 | 542 |
| | altitude | 06/05/2005 | P4 | kresoxim-methyl | 9 | 63 | 542 |
| | altitude | 06/05/2005 | P4 | fenpropimorphe | 9 | 75 | 650 |
| | sphère | 15/04/2005 | P2 | cyproconazole | 9 | 94 | 852 |
| | sphère | 15/04/2005 | P2 | trifloxystrobine | 9 | 40 | 364 |
| | altitude | 06/05/2005 | P2 | epoxiconazole | 9 | 63 | 568 |
| | altitude | 06/05/2005 | P2 | kresoxim-methyl | 9 | 63 | 568 |
| | altitude | 06/05/2005 | P2 | fenpropimorphe | 9 | 75 | 682 |

**Table A 8: 2004-2005 pesticides applications**

| Type | Product | Date | Application dose | unit of application dose | Plot | Active ingredient (A.I.) | Concentration of A.I | unit of A.I. | Area of application (ha) | Applied dose (g/ha) | Total applied mass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbicide | novall | 28/08/2003 | | | P5 | quinmerac | | | 16 | 200 | 3208 |
| | novall | 28/08/2003 | | | P5 | metazachlor | | | 16 | 800 | 12832 |
| | Roundup | 20/08/2003 | | | P5 | glyphosate | | | 16 | 1080 | 17323 |
| | Stratos | 15/09/2003 | | | P5 | cycloxydime | | | 16 | 120 | 1925 |
| | chlortoluron | 15/10/2003 | | | P1 | chlorotoluron | | | 10 | 2549 | 25488 |
| | Célio | 10/11/2003 | | | P1 | clodinafop-propargyl | | | 10 | 42 | 425 |
| | Célio | 10/11/2003 | | | P1 | cloquintocet-mexyl | | | 10 | 11 | 106 |
| | Atlantis | 05/04/2004 | | | P1 | mesosulfuron methyl | | | 10 | 11 | 106 |
| | Atlantis | 05/04/2004 | | | P1 | iodosulfuron-methyl | | | 10 | 2 | 21 |
| | Colzor trio | 28/08/2003 | | | P4 | napropamide | | | 9 | 656 | 5690 |
| | Colzor trio | 28/08/2003 | | | P4 | clomazone | | | 9 | 105 | 910 |
| | Colzor trio | 28/08/2003 | | | P4 | dimethachlor | | | 9 | 656 | 5690 |
| | Roundup | 20/08/2003 | | | P4 | glyphosate | | | 9 | 1080 | 9364 |
| | Pilot | 15/09/2003 | | | P4 | quizalofop ethyl D | | | 9 | 60 | 520 |
| | Stratos | 10/03/2004 | | | P4 | cycloxydime | | | 9 | 200 | 1734 |
| | Nikeyl | 05/05/2004 | | | P2 | aclonifen | | | 9 | 1400 | 12726 |
| | Nikeyl | 05/05/2004 | | | P2 | flurtamone | | | 9 | 376 | 3418 |
| | Roundup | 04/04/2004 | | | P2 | glyphosate | | | 9 | 1080 | 9817 |
| | chlortoluron | 15/10/2003 | | | P3 | chlorotoluron | | | 7 | 1800 | 12096 |
| | Célio | 10/11/2003 | | | P3 | clodinafop-propargyl | | | 7 | 30 | 202 |
| | Célio | 10/11/2003 | | | P3 | cloquintocet-mexyl | | | 7 | 8 | 50 |
| | Atlantis | 05/04/2004 | | | P3 | mesosulfuron methyl | | | 7 | 8 | 50 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Atlantis | 05/04/2004 | P3 | iodosulfuron-methyl | 7 | 2 | 10 |
| Fungicide | Opéra | 20/04/2004 | P1 | pyraclostrobine | 10 | 75 | 753 |
| | Opéra | 20/04/2004 | P1 | epoxiconazole | 10 | 28 | 283 |
| | Altitude | 12/05/2004 | P1 | epoxiconazole | 10 | 89 | 885 |
| | Altitude | 12/05/2004 | P1 | kresoxim-methyl | 10 | 89 | 885 |
| | Altitude | 12/05/2004 | P1 | fenpropimorphe | 10 | 106 | 1062 |
| | Opéra | 20/04/2004 | P3 | pyraclostrobine | 7 | 53 | 358 |
| | Opéra | 20/04/2004 | P3 | epoxiconazole | 7 | 20 | 134 |
| | Altitude | 12/05/2004 | P3 | epoxiconazole | 7 | 63 | 420 |
| | Altitude | 12/05/2004 | P3 | kresoxim-methyl | 7 | 63 | 420 |
| | Altitude | 12/05/2004 | P3 | fenpropimorphe | 7 | 75 | 504 |
| Insecticide | Karaté Zéon | 10/09/2003 | P5 | lambda-cyhalothrin | 16 | 5 | 80 |
| | Karaté Zéon | 05/03/2004 | P5 | lambda-cyhalothrin | 16 | 5 | 80 |
| | Aztec | 02/06/2004 | P5 | triazamate | 16 | 28 | 449 |
| | Karaté K | 02/06/2004 | P1 | lambda-cyhalothrin | 10 | 7 | 71 |
| | Karaté K | 02/06/2004 | P1 | pirimicarb | 15 | 93 | 1416 |
| | Karaté Zéon | 10/09/2003 | P4 | lambda-cyhalothrin | 9 | 5 | 43 |
| | Karaté Zéon | 05/03/2004 | P4 | lambda-cyhalothrin | 9 | 5 | 43 |
| | Aztec | 02/06/2004 | P4 | triazamate | 9 | 28 | 243 |
| | Karaté K | 02/06/2004 | P3 | lambda-cyhalothrin | 7 | 5 | 34 |
| | Karaté K | 02/06/2004 | P3 | pirimicarb | 7 | 100 | 672 |

**Table A 9: 2003-2004 pesticides applications**

| Type | Product | Date | Application dose | unit of application dose | Plot | Active ingredient (A.I.) | Concentration of A.I | unit of A.I. | Area of application (ha) | Applied dose (g/ha) | Total applied mass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbicide | Roundup | 25/09/2002 | | | P5 | glyphosate | | | 16 | 720 | 11549 |
| | isoproturon | 05/11/2002 | | | P5 | isoproturon | | | 16 | 1200 | 19248 |
| | Baghera | 05/03/2003 | | | P5 | fenoxaprop-p-ethyl | | | 16 | 40 | 642 |
| | Baghera | 05/03/2003 | | | P5 | diclofop-methyl | | | 16 | 500 | 8020 |
| | Baghera | 05/03/2003 | | | P5 | mefenpyr-diethyl | | | 16 | 80 | 1283 |
| | Roundup | 15/08/2002 | | | P1 | glyphosate | | | 14 | 720 | 10195 |
| | Tréflan | 15/09/2002 | | | P1 | trifluraline | | | 14 | 960 | 13594 |
| | novall | 15/09/2002 | | | P1 | quinmerac | | | 14 | 200 | 2832 |
| | novall | 15/09/2002 | | | P1 | metazachlor | | | 14 | 800 | 11328 |
| | isoproturon | 25/10/2002 | | | P4 | isoproturon | | | 9 | 1200 | 10404 |
| | Célio | 15/11/2002 | | | P4 | clodinafop-propargyl | | | 9 | 30 | 260 |
| | Célio | 15/11/2002 | | | P4 | cloquintocet-mexyl | | | 9 | 8 | 65 |
| | kino | 15/04/2003 | | | P4 | MCPP | | | 9 | 260 | 2254 |
| | kino | 15/04/2003 | | | P4 | DCPP | | | 9 | 620 | 5375 |
| | kino | 15/04/2003 | | | P4 | 2,4-MCPA | | | 9 | 320 | 2774 |
| | Roundup | 15/09/2002 | | | P2 | glyphosate | | | 9 | 540 | 4909 |
| | chlortoluron | 15/10/2002 | | | P2 | chlorotoluron | | | 9 | 2000 | 18180 |
| | Célio | 15/11/2002 | | | P2 | clodinafop-propargyl | | | 9 | 30 | 273 |
| | Célio | 15/11/2002 | | | P2 | cloquintocet-mexyl | | | 9 | 8 | 68 |
| | Nikeyl | 15/05/2003 | | | P3 | aclonifen | | | 7 | 1225 | 8232 |
| | Nikeyl | 15/05/2003 | | | P3 | flurtamone | | | 7 | 329 | 2211 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Fungicide | unix | 15/04/2003 | P5 | cyprodinil | 16 | 300 | 4812 |
| | amistar | 15/04/2003 | P5 | azoxystrobine | 16 | 100 | 1604 |
| | unix | 15/05/2003 | P5 | cyprodinil | 16 | 300 | 4812 |
| | amistar | 15/05/2003 | P5 | azoxystrobine | 16 | 100 | 1604 |
| | éria | 15/12/2002 | P1 | difenoconazole | 14 | 125 | 1770 |
| | éria | 15/12/2002 | P1 | carbendazime | 14 | 250 | 3540 |
| | calidan | 15/04/2003 | P1 | iprodione | 14 | 350 | 4956 |
| | calidan | 15/04/2003 | P1 | carbendazime | 14 | 175 | 2478 |
| | opéra | 15/04/2003 | P4 | pyraclostrobine | 9 | 93 | 807 |
| | opéra | 15/04/2003 | P4 | epoxiconazole | 9 | 35 | 303 |
| | altitude | 15/05/2003 | P4 | epoxiconazole | 9 | 50 | 434 |
| | altitude | 15/05/2003 | P4 | kresoxim-methyl | 9 | 50 | 434 |
| | altitude | 15/05/2003 | P4 | fenpropimorphe | 9 | 60 | 520 |
| | Ogam | 25/04/2003 | P2 | epoxiconazole | 9 | 75 | 682 |
| | Ogam | 25/04/2003 | P2 | kresoxim-methyl | 9 | 75 | 682 |
| Insecticide | Karaté Zéon | 15/03/2003 | P1 | lambda-cyhalothrin | 14 | 5 | 71 |
| | Karaté Zéon | 15/06/2003 | P4 | lambda-cyhalothrin | 9 | 5 | 43 |

**Table A 10: 2002-2003 pesticides applications**

## Appendix IV Design of experiments and detailed uncertainty analysis to develop and validate a solid-phase microextraction/gas chromatography–mass spectrometry method for the simultaneous analysis of 16 pesticides in water

*Preliminary notes:*

*(1) This work was initiated through Tanya Culhaoglu's 6-months master project and further 3-months stay at the Cemagref of Antony. The paper itself was made possible thanks to the close involvement of each of the six co-authors.*

*(2) The paper was published in the Journal of Chromatography A, vol 1217(33), 5317-5327, 2010.*

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Abstract
A solid-phase microextraction (SPME)/gas chromatography (GC)–mass spectrometry (MS) multiresidue analytical method was developed for 16 pesticides presenting different physicochemical properties including diphenyl ether, triazine, ureas, acetamides, benzofuran, thiocarbamate, pyridine carboxamides, chloronitrile, piperedine, and azoles. Optimization was achieved by means of the Design of Experiments methodology. Extraction temperature, extraction time, desorption temperature, and NaCl addition were the factors exhibiting the most significant effects on pesticide extraction. Validation was carried out through model adequacy and specificity tests, limits of quantification and detection determination, and full uncertainty assessment on the whole analytical method. Good first- and second-order model adequacy was found for pesticide calibration. LOQs were in the 0.05–0.5 µg/L range and specificity recoveries varied from 75 to 140%. These results were considered acceptable for our research purposes on highly concentrated agricultural flows. Uncertainty calculations accounted for several steps: standard preparation, calibration model selection, and use. On average, real sample concentration uncertainties were lower than 10%, indicating that the analytical method performed very well. Its application to 61 real water samples confirmed the presence of some pesticide concentrations in relation to farmer use, whereas other molecules were usually either not detected or not quantified.

Key words: design of experiments, uncertainties, validation, SPME, GCMS, pesticides.

## INTRODUCTION

The quality of natural water bodies may be affected by pesticide inputs resulting from their use and transfer from urbanized or agricultural areas. These molecules are suspected to impact ecosystems and human health [1-3]. The Water Framework Directive (WFD; 2000/60/EC) [4] is a major legislative effort aiming at protecting European water bodies, requiring that they reach a "good ecological" status by 2015. The WFD focuses on several pollutants including pesticides. To comply with this regulation, maximum authorized concentrations in raw waters of 2 $\mu g.L^{-1}$ per pesticide and 5 $\mu g.L^{-1}$ for all pesticides have been established. To attain this objective, article 16 of the WFD states that monitoring protocols have to be developed to detect and quantify pesticides. Such analytical methods should be simple, cheap, fast, and able to simultaneously detect trace concentrations of several molecules. Multiresidue analyses are increasingly common and their development has already been published [5-8]. Usually single methods rarely combine the simultaneous analysis of pesticides presenting varying physicochemical properties. This implies that determining the pesticide concentration in water samples may require the use of several analytical methods to characterize pesticides in different families. Among existing analytical methods, gas chromatography (GC) has been widely used for pesticide analysis. Different types of detection methods can be employed such as flame ionization detection (FID), ultraviolet, (UV), diode area detection (DAD), and mass spectrometry (MS). The GC–MS association has shown high sensitivity and specificity for pesticide analysis [9-12]. However, it was shown that GC–MS was not totally adequate for phenoxy acids such as 2,4-D [13-15] and thermolabile phenylurea herbicides (PUHs). When analyzed by GC, PUHs can decompose to isocyanates, carbamates, or anilines depending on the selected injection solvent [16]. Derivatization procedures can help enhance the determination of PUHs by GC [17]. However, Peña et al. (2002) developed a GC–MS method for seven phenylureas [18] that did not include a derivatization step. For these molecules, other analytical techniques are sometimes better adapted, such as liquid chromatography (LC) coupled with UV, diode area detection, or mass spectrometer [19]. For instance, the LC-MS combination showed good results for ureas and carbamates [19, 20], as well as molecules such as prochloraz and bentazon [20-23].

Pesticide concentrations in surface waters are usually very small. An extraction step is therefore needed prior to their analysis. Liquid–liquid extraction (LLE) has been used to detect organic micropollutants in liquid samples but requires a large volume of solvents. This technique was subsequently largely replaced by solid-phase extraction (SPE). SPE is less solvent- and time-consuming than the LLE extraction technique because several samples can be extracted simultaneously. More recently, new extraction techniques involving simpler, faster, less costly, and solvent-free procedures have been introduced [25, 26] such as solid-phase micro-extraction (SPME) [24] and stir-bar sorptive extraction (SBSE) [25]. SBSE sensitivity is generally higher than that of SPME [25]. However, SBSE is still mainly limited to polydimethylsiloxane (PDMS) coatings, whereas SPME fibers are available on a wider range of material. In addition, SPME has the advantage of being easily automated, whereas SBSE automation is still being developed [26, 27]. In both SPME and SBSE techniques, a fused silica fiber coated with a solid phase is prone to adsorb the compounds until equilibrium is reached with the surrounding sample matrix [28]. This step is dependent on the pesticide's physicochemical characteristics. Subsequent to extraction, the fiber or the stir-bar is transferred into the injection port of the GC, where the analytes are desorbed. SPME has been successfully employed for pesticide extraction from several matrices including water samples [6-8, 16, 29-32].

Analytical method development and optimization require setting values for several parameters that may influence the method's efficiency. In extraction procedures, several factors have been identified as potentially impacting pesticide extracted amounts: extraction and desorption temperatures [33] and times [34, 35], pH [8, 29, 36], ionic strength (via salt addition) [37], stirring speed [38], and fiber coating [8, 30]. Optimization based on the variation of one variable at a time (OVAT) has often been used. However, it is time-consuming and may produce misleading conclusions. Indeed, the variables to be optimized are usually not totally independent [39]. The Design of Experiments (DoE) methodologies help to optimize a process by varying identified factors at the same time in a cautious and programmed way [40]. It also enables possible interactions among factors to be taken into account in only a few experiments compared to the OVAT methodology. According to the selected DoE resolution, for some factors, the main effects may be confused with some interactions. A careful analysis of the results is therefore needed.

Once developed, a method has to be carefully evaluated. This is commonly done by assessing calibration model adequacy, determining limits of quantification and detection, and testing the method's repeatability and specificity [7, 29, 33, 41]. A result obtained from an analytical method cannot be totally reliable if not given with its associated uncertainty. However, detailed uncertainty calculations have rarely been provided, thereby impoverishing the overall method evaluation [42]. Normalized guidelines exist that help implement these validation steps [43, 44].

The present work focuses on an SPME GC–MS combination as an analytical method for the simultaneous analysis of 16 pesticides presenting varying physicochemical characteristics. The pesticides were selected based on their frequency and level of quantification in surface waters coming from agricultural watersheds [45]. No discrepancy among molecules was detected, giving a set of pesticides that are usually not analyzed simultaneously. For research needs, a single rapid method for the selected molecules was desired to further evaluate pesticide transfer through agricultural drainage and wetland outflows. The objectives were: (1) to develop and optimize a multiresidue method implementing experimental designs; (2) to validate this method following common required criteria, and (3) to provide a step-by-step detailed uncertainty calculation for a full assessment of the method.

## MATERIAL

*Chemicals and solutions*

The 16 pesticides (Table A 1) of at least 97.0% purity included in this study were isoproturon (IPU), chlorotoluron (CTU), atrazine (ATZ), chlorothalonil (CTL), prosulfocarb (PSC), ethofumesate (ETF), fenpropidin (FPP), *s*-metolachlor (MTL), metazachlor (MTZ), napropamide (NPP), cyproconazole (CYP), aclonifen (ACF), diflufenican (DFF), mefenpyr-diethyl (MFP), epoxiconazole (EPX), and tebuconazole (TBC). Three other pesticides (2,4-D, bentazon, and prochloraz) had also been tested but could not be directly analyzed after direct injection tests. Derivatization procedures may be necessary for these molecules [15, 17]. They were therefore not included in the multiresidue analytical method. Analytical standard Pestanal and acetone SupraSolv were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). A stock solution including the 16 molecules was prepared every 3 months by weighing approximately 0.75 mg of each compound and diluting them into acetone to obtain a 50-mg.$L^{-1}$ concentration solution. Intermediate solutions (500 µg.$L^{-1}$) were made in acetone every month by diluting the stock solution. Both the stock and the intermediate solutions were stored at −20°C. Finally, six standards whose target concentrations were 0.05, 0.1, 0.5, 1, 2, and 5 µg.$L^{-1}$ were prepared from the intermediate solution in glass bottle mineral water (Evian, France) before each series of analyses to obtain the calibration curves. NaCl salt was obtained from Alfa Aesar GmbH (Karlsruhe, Germany).

| Pesticide | Abbreviation | Purity (%) | Retention time (min) | Quantification ions (m/z) | Qualification ions (m/z) | | Target ratio | |
|---|---|---|---|---|---|---|---|---|
| | | | | IQT | IQL1 | IQL2 | $\frac{IQL_1}{IQT}$ | $\frac{IQL_2}{IQT}$ |
| Isoproturon | IPU | 99.9 | 9.28 | 146 | 161 | - | 23.6 | - |
| Chlorotoluron | CTU | 99.7 | 9.42 | 132 | 167 | 169 | 37.5 | 10.6 |
| Atrazine | ATZ | 97.5 | 15.90 | 200 | 58 | 215 | 53.6 | 68.2 |
| Chlorothalonil | CTL | 99.3 | 16.69 | 266 | 264 | 268 | 77.5 | 49.0 |
| Prosulfocarb | PSC | 99.1 | 18.55 | 91 | 86 | 65 | 74.4 | 16.3 |
| Fenpropidin | FPP | 97.3 | 18.67 | 98 | 99 | - | 7.3 | - |
| Ethofumesate | ETF | 99.5 | 18.73 | 161 | 137 | 179 | 70.8 | 44.7 |
| *s*-metolachlor | MTL | 97.6 | 19.19 | 162 | 238 | 146 | 35.1 | 14.8 |
| Metazachlor | MTZ | 99.9 | 20.52 | 81 | 133 | 132 | 95.4 | 72.5 |
| Napropamide | NPP | 99.8 | 23.48 | 72 | 100 | 127 | 19.2 | 11.1 |
| Cyproconazole | CYP | 99.8 | 24.19 | 139 | 222 | 125 | 119.7 | 52.8 |
| Aclonifen | ACF | 99.8 | 25.31 | 77 | 264 | 194 | 70.7 | 46.4 |
| Diflufenican | DFF | 97.0 | 27.82 | 266 | 246 | 394 | 10.8 | 12.1 |
| Tebuconazole | TBC | 99.6 | 28.84 | 125 | 70 | 83 | 84.0 | 61.5 |
| Epoxiconazole | EPX | 99.2 | 28.60 | 192 | 138 | 165 | 34.0 | 37.4 |
| Mefenpyr-diethyl | MFP | 99.7 | 28.62 | 253 | 255 | 227 | 68.1 | 34.3 |

**Table A 11: Retention time, purity and typical fragment ions for the 16 pesticides used in this SPME GC-MS method.**

*Extraction procedure*

The following materials were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France): 100 µm polydimethylsiloxane (PDMS), 85 µm polyacrylate (PA), 50 µm divinylbenzene/carbowax/polydimethylsiloxane (DVB/CAR/PDMS), and 65 µm PDMS/DVB SPME fibers. The fibers were conditioned before use in the GC-injector port for 0.5, 1, or 2 h in temperatures ranging from 250°C to 300°C according to the supplier's instructions. The fiber selection was based on its adsorption capacity. Four solutions containing all studied pesticides at a concentration of 500 µg $L^{-1}$ were prepared in 20-mL amber glass vials containing 18 mL of ultrapure water (ELGA LabWater, Veolia Water STI, Antony, France). These solutions were analyzed according to parameters proposed by Sauret-Szczepanski et al. (2006) [31], who developed an SPME GC–MS method including some of the pesticides used in our study. These parameters were set as follows: extraction time = 40 min, desorption time = 5 min, extraction temperature = 50°C, desorption temperature = 270°C, stirring speed = 500 rpm. The pH was not adjusted and the ionic strength was not corrected.

*GC-MS*

The GC–MS apparatus was a Trace/DSQ model from Thermo Fisher Scientific (Les Ulis, France) equipped with an LHX PAL front-end automation system (Thermo Fisher Scientific, Les Ulis, France). X-Calibur and Cycle Composer software was used to control and acquire data from the GC–MS and CombiPAL machines, respectively. Pesticide separation was conducted through a Zebron ZB-5MS capillary column (60 m, 0.25 mm ID, 0.25 µm df) from Phenomenex (Le Pecq, France) using helium as a carrier gas (1.3 mL/min). The splitless injection mode was selected because it was adapted to highly diluted compounds. Ionization

was carried out in the mass spectrometer under vacuum by electron impact with a 70-eV ionization energy.

## METHOD

*Development and optimization*

Samples were analyzed using the following oven temperature program: initial temperature 60°C (held for 1 min), 18°C/min to 160°C (held for 1 min), 8°C/min to 230°C (held for 1 min), and finally 2°C/min to 280°C (held for 5 min). Transfer line, injector, and source temperatures were set to 250, 270, and 250°C, respectively. The SPME GC–MS method optimization was carried out according to the DoE methodology. Seven factors (Table A 12) were identified as possibly affecting the response variables, namely, each pesticide's peak area.

| Level | | Extraction Temperature | Extraction Time | Desorption Temperature | Desorption Time | Stirring Speed | pH | [NaCl] |
|---|---|---|---|---|---|---|---|---|
| | | (°C) | (min) | (°C) | (min) | (rpm) | (-) | (%)[(a)] |
| −1 | | 30 | 30 | 200 | 5 | 250 | 5 | 0 |
| 0 | | 45 | 55 | 235 | 10 | 500 | 7 | 10 |
| +1 | | 60 | 80 | 270 | 15 | 750 | 9 | 20 |
| Experiments | Block | | | | | | | |
| 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1 | −1 | −1 | +1 | −1 | +1 | +1 | −1 |
| 3 | 1 | +1 | +1 | +1 | −1 | −1 | −1 | −1 |
| 4 | 1 | −1 | −1 | −1 | +1 | +1 | +1 | +1 |
| 5 | 1 | +1 | +1 | −1 | +1 | −1 | −1 | +1 |
| 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 2 | −1 | +1 | −1 | +1 | −1 | +1 | −1 |
| 8 | 2 | +1 | −1 | −1 | +1 | +1 | −1 | −1 |
| 9 | 2 | −1 | +1 | +1 | −1 | −1 | +1 | +1 |
| 10 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 2 | +1 | −1 | +1 | −1 | +1 | −1 | +1 |
| 12 | 3 | −1 | +1 | +1 | +1 | +1 | −1 | −1 |
| 13 | 3 | −1 | +1 | −1 | −1 | +1 | −1 | +1 |
| 14 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 3 | +1 | −1 | −1 | −1 | −1 | +1 | +1 |
| 16 | 3 | +1 | −1 | +1 | +1 | −1 | +1 | −1 |
| 17 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 4 | +1 | +1 | +1 | +1 | +1 | +1 | +1 |
| 19 | 4 | −1 | −1 | −1 | −1 | −1 | −1 | −1 |
| 20 | 4 | −1 | −1 | +1 | +1 | −1 | −1 | +1 |
| 21 | 4 | +1 | +1 | −1 | −1 | +1 | +1 | −1 |

**Table A 12: Screening DoE domain and matrix. [(a)] 100% NaCl was taken as its solubility measured in mineral water at 20°C, i.e., 360 g/L.**

Two steps were followed for the method development:

(1) Seven factors were screened by means of a fractional factorial design;

(2) A response surface design was selected to optimize the factors exhibiting the highest influence on the response variable.

In the first step, a $2^{7-3}$ fractional factorial screening DoE was chosen (resolution IV), with the addition of five central points. This notation indicates that seven factors were tested, with two levels for each factor investigated and three additional factors compared to the full base ($2^4$) DoE. This led to a total of 21 experiments conducted in four blocks, each corresponding to a single day. We assumed that any interactions greater than or equal to three were negligible. Their associated contrasts were therefore confused with the factor's main effect. Based on previous studies [8] [29], and considering the restrictions inherent to the apparatus (vial volume, maximal extraction temperature, maximal stirring speed, etc.), two levels (referred to as −1 and +1 for the lower and upper limit, respectively, of the variation range of each factor) were determined for each factor: (1) extraction temperature (25–65°C), (2) extraction time (30–80 min), (3) desorption temperature (200–270 °C), (4) desorption time (5–15 min), (5) stirring speed (250–750 rpm), (6) sample pH (acid–basic), and (7) ionic strength, through NaCl addition (0–30% of its solubility in water at 20°C taken at 360 $g.L^{-1}$). The order of the 21 experiments was randomized. Secondly, a response surface composite design was prepared to optimize the factors exhibiting the highest influence on the response variable. The JMP® software (SAS Institute Inc.) was used and provided the effects and interactions of the factors for each pesticide peak area response. Analysis of variance was used to determine the significance of factors and interactions comparing their variations to the model error. Student *t*-tests and associated *p*-values were processed. A 5% significance level was selected.

*Validation*

After optimization, the analytical method was tested following the steps of the French normalized method NF T90-210 [43] for its validation. Linearity, limits of detection (LOD) and quantification (LOQ), specificity, and repeatability were evaluated. Calibration curves were fitted by either linear or second-order polynomial models. Consequently, not only linearity but also the adequacies of the models were tested. Calibration curve adjustments were assessed by lack-of-fit tests calculating the Fisher statistics and accepting an $\alpha$ level of 1% at six concentration levels (0.05, 0.1, 0.5, 1, 2, and 5 $\mu g.L^{-1}$). Five replicates were analyzed for each standard concentration level.

Predetermined LOQs were compared with those obtained from mineral water spiked to the tested LOQ and analyzed six times under repeatability conditions. Predetermined LOQs were validated when the following two conditions were met [43, 46]:

Trueness criterion:

$$\left| \frac{LOQ - \bar{u}_{LOQ}}{\frac{\sigma_{LOQ}}{\sqrt{n}}} \right| < 10 \qquad (1.)$$

and

Precision criterion:

$$\frac{\sigma_{LOQ}}{LOQ} \cdot 100 < 20 \qquad (2.)$$

where $\bar{u}_{LOQ}$ and $\sigma_{LOQ}$ were the $n$=5 measures of mean and standard deviation, respectively.

Theoretical LODs were calculated from verified LOQs divided by three. Specificity (matrix effect) was tested by adding known amounts of pesticides (0.05, 0.1, 1, and 5 $\mu g.L^{-1}$) in two replicates to a mixture made of two types of matrices. The matrices consisted of water samples taken from two rivers presenting similar characteristics of agricultural sub-surface drainage or wetland outflows, both samples of interest for us [47]. Calculated concentrations were plotted against spiked concentrations. Specificity was validated running Student *t*-tests

($\alpha$ = 1%) to detect that the slope was not significantly different from 1 and that the coordinate origin was not significantly different from 0. Recovery rates were also calculated as the ratios between calculated and spiked concentrations, expressed as a percentage. Repeatability stability was evaluated from Cochran tests at the 1% level of significance and by calculating coefficients of variation (COVs) among five replicates of each concentration level.

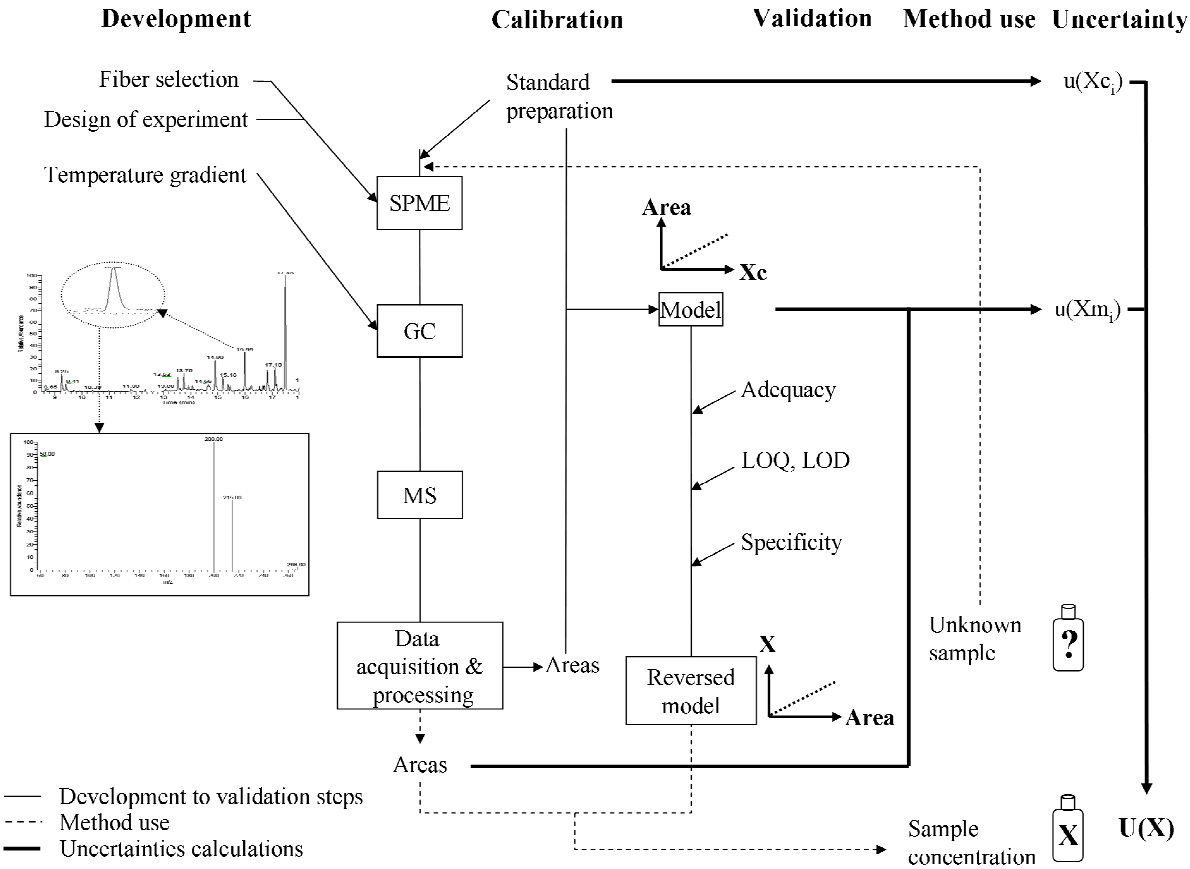


**Fig. A 1: Diagram of the analytical method development procedure**

*Uncertainties*

Once a method has been developed, uncertainties are usually evaluated by only assessing method repeatability and reproducibility on real samples. However, despite providing relevant and necessary information on these sources of variation, the variation specifically contributed by model coefficients is hidden. In the present study, we specifically assessed the uncertainty resulting from four chronologically implemented steps: (1) standard preparation, (2) calibration model determination, (3) real sample concentration determination using the reversed-postulated models for each pesticide molecule, and (4) combined expanded final uncertainty calculation. Uncertainty values are provided with two significant digits. The result is subsequently written with the same number of decimals as its uncertainty [44]. Details on uncertainty assessment are provided in the appendix and were based on the French XP T 90-220 normalization methods [43] and the EURACHEM/CITAC guide [44]. A flow-diagram summarizing the whole procedure is shown in Fig. A 1.

## RESULTS AND DISCUSSION

*Fiber selection*

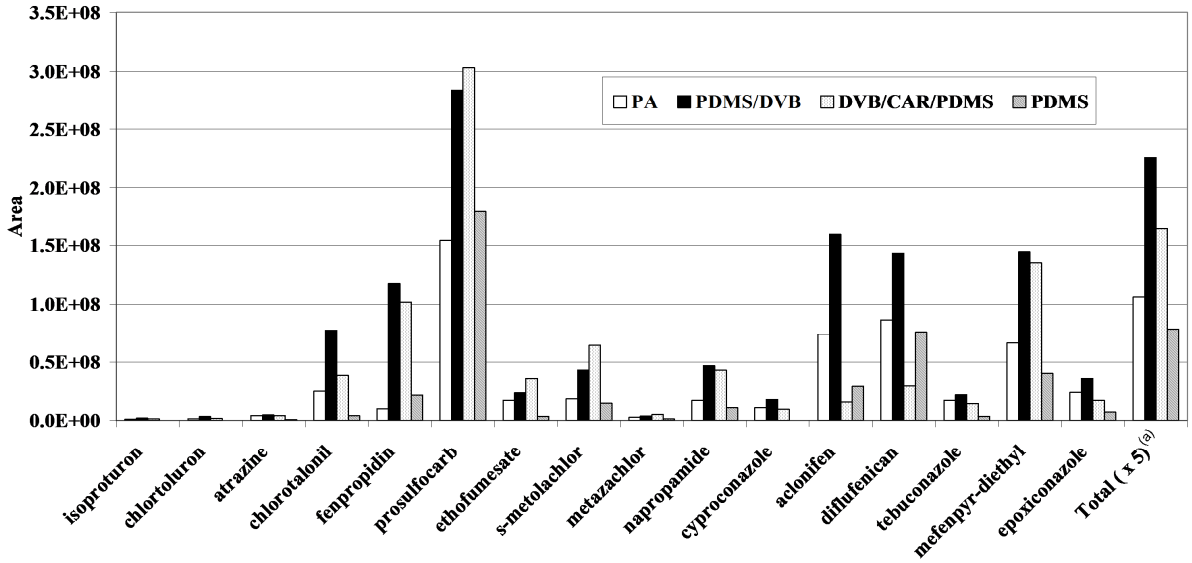


**Fig. A 2: Peak areas for each pesticide on the four different fibers. (a) The total cumulated area is divided by five to better fit in the graph.**

The pesticide chromatogram areas and cumulated areas of the 16 pesticides are reported in Fig. A 2. Overall, PA-coated fibers did not lead to significant responses. The highest extraction efficiency was shown with DVB/CAR/PDMS for prosulfocarb, ethofumesate, *s*-metolachlor, and metazachlor. However, the PDMS/DVB association resulted in the highest areas for the other 12 pesticides. This is in agreement with previous studies where PDMS/DVB coating was selected to extract metolachlor [7, 8, 29], chlorotalonil, and atrazine [7, 8]. Three additional pesticides (deethylatrazine, deisopropylatrazine, and quinmerac Pestanal obtained from Sigma-Aldrich, Saint Quentin Fallavier, France) were tested with the other 16 molecules. They were not extracted by any of the PDMS fibers and a very poor response was found on the most polar fiber (PA). This may be explained by these molecules presenting very low hydrophobicity properties.

*Method development and optimization*

Fractional factorial design of experiment

| Pesticide | Extraction Temperature (°C) | Extraction Time (min) | Desorption Temperature (°C) | Desorption Time (min) | Stirring Speed (rpm) | pH (-) | NaCl (%) |
|---|---|---|---|---|---|---|---|
| IPU | 0.0049 (−) | 0.0440 (+) | 0.0165 (+) | | | | 0.0049 (+) |
| CTU | 0.0011 (−) | | 0.0011 (+) | | 0.0259 (−) | | 0.0012 (+) |
| ATZ | 0.0302 (−) | 0.0329 (+) | 0.0026 (+) | | | | 0.0051 (+) |
| CTL | 0.0003 (−) | 0.0101 (+) | 0.0031 (+) | | | | 0.0147 (+) |
| PSC | | | | | | | |
| FPP | | | | | | 0.0082 (+) | |
| ETF | 0.0025 (−) | | 0.0016 (+) | | | | 0.0097 (+) |
| MTL | | | 0.0031 (+) | | | | 0.0034 (+) |
| MTZ | | | 0.0067 (+) | | | | |
| NPP | | | 0.0027 (+) | | | | 0.0271 (+) |
| CYP | | | 0.0024 (+) | | | | |
| ACF | | | | | | | |
| DFF | | | 0.0438 (+) | | | | |
| TBC | | | | | | | |
| EPX | | | | | | | |
| MFP | | | 0.0184 (+) | | | | |

**Table A 13: Design of experiments *p*-values for significant positive (+) or negative (−) effects.**

*Desorption time and stirring speed*

The significant effects of the various factors are presented in Table A 13. Among the seven factors, desorption time and stirring speed had no influence on pesticide chromatogram area responses. An exception was noted with CTU, for which a significant negative ($p$ = 0.0259) influence of stirring speed was observed. However, Llompart et al. (1998) [38] have shown that extraction yield increased when increasing stirring speed. Stirring limits the diffusion boundary layer thickness. This result on CTU therefore may not be considered correct. Indeed, when carrying out several tests for one factor, an incorrect significant positive or negative effect of the factor may be found. Considering a 5% level of significance in each experiment, it should be noted that on average, 1 out of 20 experiments may lead to an erroneous result. Desorption time and stirring speed were finally set to 10 min and 500 rpm, respectively. Rocha et al. (2008) [34] showed that a 15-min desorption time was long enough to properly desorb most pesticides.

*pH*

Only FPP showed a positive response to pH ($p$=0.0082). This molecule presents a pKa of 10.13 [48], thus placing it as a weak base. Its solubility significantly decreases with increasing pH [49]. A significant increase in pH would lead to a higher proportion of an FPP neutral form, which has a lower solubility and thus a higher affinity for the PDMS/DVB fiber than those of its cationic form. A significant effect of pH on ionizable pesticide was found by Beltran et al. (2000) [36]. Our results showed that pH was not significant for the other molecules, since most of them were not ionizable, as was also found in previous studies [8, 29]. It was therefore decided not to change the water sample pH in the analytical method.

*Desorption temperature*

For SPME procedures, desorption temperature should be high enough to properly desorb previously extracted molecules but without damaging the fiber. This factor had a significant positive effect on 11 pesticides. This was demonstrated by a higher extraction for

270°C than for 200°C. In addition, this high temperature did not affect thermosensitive molecules such as IPU. This factor was set to 270°C, because the supplier advocated not using the fiber above this temperature.

*NaCl*

Salt addition significantly enhanced pesticide extraction for seven soluble molecules (Table A 13). It did not show any significant effect on MFP, ACF, DFF, PSC, and FPP, whose solubility were 20 $mg.L^{-1}$ (20°C), 2.5 $mg.L^{-1}$ (20°C), <0.05 $mg.L^{-1}$ (25°C), 13.2 $mg.L^{-1}$ (20°C), and 530 $mg.L^{-1}$ (20°C), respectively [49]. These solubilities were therefore low. Moreover, considering that they were among the most hydrophobic of the set of 16 pesticides, their affinity for the fiber was higher than that of the other molecules. It was suggested that the movement of the least polar molecules toward the fiber is reduced when the ionic strength is increased [37]. However, no significant negative effect was observed from these experiments. Extraction of apolar pesticides (e.g., DFF, PSC, NPP) was therefore not affected by salt addition. Consequently, salt addition helped in extracting IPU ($p$=0.0049), CTU ($p$=0.0012), ATZ ($p$=0.0051), CTL ($p$=0.0147), ETF ($p$=0.0097), MTL ($p$=0.0034), and NPP ($p$=0.0271), thus increasing these pesticide affinities for the PDMS/DVB fiber, as also found in previous studies [50].

*Extraction time*

Extraction time had a significant positive effect on IPU ($p$=0.0440), ATZ ($p$=0.0329), and CTL ($p$=0.0101). Arthur and Pawliszyn (1990) [24] showed that SPME was based on an equilibrium process between the liquid phase and the coating solid phase. This process could be very long depending on the molecule's diffusion into the solid phase, which should explain these results.

*Extraction temperature*

High extraction temperatures showed significant negative effects on IPU ($p$=0.0049), CTU ($p$=0.0011), ATZ ($p$=0.0302), CTL ($p$=0.0003), and ETF ($p$=0.0025). This was also noted by previous authors [31, 51] and may be explained by the molecule stability and competition between adsorption and desorption kinetics on the fiber. The extraction temperature was therefore set to 45°C, which was a trade-off between proper extraction and pesticide degradation.

*Block and interaction*

Significant block effects were only found for IPU, CTU, and CTL. These molecules may be particularly sensitive to environmental factors compared to the other 13. Significant negative interactions were only highlighted for the extraction of CTU, CTL, and ETF. As no other significant interaction was demonstrated, it was considered that the method could be optimized on the entire set of 16 molecules in spite of these results.

Optimization

A composite surface response DoE was implemented to help optimize extraction time and NaCl addition. For both factors showing significant positive effects on the response, the experimental domain was moved toward larger values. Five extraction times (20, 30, 55, 80, and 90 min) and NaCl addition levels (0, 15, 45, 80, and 90%) were selected in 11 experiments. However, salt crystallization was observed on the fiber, which broke after the eighth experiment was completed. Additional tests were conducted, concluding that a 30% NaCl addition was the maximal acceptable rate to obtain fairly good extraction of most pesticides without damaging the fiber. The remaining results of the incomplete response surface design showed that, overall, the response increase after 55 min was not as significant as that between 20 and 55 min, except for ETF, NPP, MFP, and MTL. These compounds are fairly soluble and hydrophobic and salt addition may not be sufficient to help them quickly sorb on the fiber. Therefore, 55 min was considered the best compromise between a reasonable extraction time and a correct response. It was concluded that the best general

conditions to quantitatively analyze the 16 pesticides simultaneously were obtained with a PDMS/DVB fiber, 45°C extraction temperature, 270°C desorption temperature, 10-min desorption time, 500-rpm stirring speed, unmodified pH, 55-min extraction time, and 30% NaCl addition.

*Method validation*

Model adequacy, LOQ, LOD

| Pesticides | Order | Range | $r^2$ | LOQ evaluation | | | LOD | Specificity | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | LOQ | Trueness | Precision | | t-value | Slope | Origin | Recovery | SD |
| | | ($\mu g.L^{-1}$) | | ($\mu g.L^{-1}$) | | (%) | ($\mu g.L^{-1}$) | | | | % | % |
| IPU | 2 | 0.05−5 | 0.9997 | 0.05 | 0.79 | 17.50 | 0.02 | 3.169 | 9.13 | 0.465 | 130.0 | 11.2 |
| CTU | 2 | 0.10−5 | 0.9992 | 0.10 | 1.60 | 22.00 | 0.03 | 3.707 | 10.44 | 0.405 | 142.0 | 9.0 |
| ATZ | 1 | 0.05−2 | 0.9987 | 0.05 | 1.97 | 12.00 | 0.02 | 3.169 | 21.92 | 1.356 | 143.0 | 13.6 |
| CTL | 2 | 0.50−5 | 0.9994 | 0.50 | 9.70 | 4.00 | 0.17 | 3.707 | 6.31 | 0.820 | 76.4 | 6.6 |
| PSC | 1 | 0.05−5 | 0.9981 | 0.05 | 5.86 | 8.90 | 0.02 | 2.977 | 24.97 | 0.520 | 120.2 | 8.9 |
| FPP | 2 | 0.05−5 | 0.9993 | 0.05 | 5.16 | 15.30 | 0.02 | 2.977 | 17.91 | 0.580 | 101.9 | 21.0 |
| ETF | 1 | 0.10−5 | 0.9985 | 0.10 | 0.70 | 8.80 | 0.03 | 3.169 | 1.00 | 2.638 | 114.9 | 11.5 |
| MTL | 1 | 0.05−5 | 0.9996 | 0.05 | 2.68 | 8.70 | 0.02 | 2.977 | 1.24 | 2.055 | 114.7 | 8.6 |
| MTZ | 1 | 0.10−5 | 0.9985 | 0.10 | 0.60 | 11.50 | 0.03 | 3.106 | 8.74 | 0.716 | 119.2 | 8.1 |
| NPP | 1 | 0.05−5 | 0.9967 | 0.05 | 4.66 | 11.00 | 0.02 | 2.977 | 3.48 | 1.095 | 106.3 | 6.3 |
| CYP | 1 | 0.50−5 | 0.9984 | 0.50 | 4.80 | 9.40 | 0.17 | 3.707 | 1.70 | 2.143 | 126.6 | 16.4 |
| ACF | 2 | 0.10−2 | 0.9993 | 0.10 | 8.30 | 9.90 | 0.03 | 3.707 | 7.12 | 2.044 | 98.1 | 15.4 |
| DFF | 2 | 0.05−5 | 0.9994 | 0.05 | 0.46 | 27.70 | 0.02 | 2.977 | 0.85 | 0.044 | 94.4 | 7.5 |
| TBC | 2 | 0.10−5 | 0.9995 | 0.10 | 6.70 | 12.90 | 0.03 | 3.169 | 4.68 | 0.455 | 101.7 | 21.3 |
| EPX | 2 | 0.10−2 | 0.9991 | 0.10 | 1.60 | 19.90 | 0.03 | 3.707 | 4.44 | 0.074 | 123.0 | 10.7 |
| MFP | 2 | 0.05−5 | 0.9992 | 0.05 | 2.56 | 8.50 | 0.02 | 2.977 | 0.06 | 0.809 | 94.3 | 16.6 |

**Table A 14: Calibration model order, validation range, correlation coefficients, limits of quantification and associated trueness and precision, limits of detection and specificity.**

Among the 16 pesticides, seven followed linear models and nine were fitted by second-order models (Table A 14). The ranges for which Fisher tests passed are presented in Table A 14. For most of the pesticides, five of the six concentration levels belonged to the validated range. ACF, EPX, CTL, and CYP were validated on a smaller range (four concentration levels among the six tested). The model correlation coefficients were high (> 0.9960). Since our research needs did not require very low LOQs, the lowest tested LOQ was 0.05 µg/L. Most of the predetermined LOQs passed the trueness and precision tests for the selected 10 and 20% criteria, respectively. However, DFF and CTU exceeded the 20% precision criterion. The highest LOQs were generally associated with the most polar molecules (ATZ, MTZ, CTU, FPP, CYP). The affinity of such pesticides for the PDMS/DVB extraction fiber, in spite of salt addition, was lower than that of more hydrophobic compounds. This had already been suggested for simazine [52]. LOQs were not at the lowest tested value for half of the pesticides. The objective of this study was to include pesticides detected in agricultural flows presenting varying characteristics using a single analytical method. Consequently, since some of them were moderately extracted, a compromise was needed between LOQ values and the multiresidue method objective.

Specificity

From calculated versus spiked concentration graphs and associated statistics, specificity assessment showed that the difference between zero and the coordinate origin could be made for all pesticides. However, the slope criterion was statistically significant for only five pesticides (ETF, MTL, CYP, DFF, MFP). Poor results were found for the slope for the remaining molecules. The calculated amounts were usually higher than the spiked amounts, with recovery values ranging from 76.4 ± 6.6% to 143.0 ± 13.6%, usually higher than 100% (Table 4). The highest recovery rates were found for IPU (130.0 ± 11.2%), CTU (142.6 ± 9.0%), and ATZ (143.0 ± 13.6%). These results should be carefully studied in concomitance with the uncertainties presented in the next section.

*Determining uncertainties*

As the optimal conditions were determined and the analytical method validated, the uncertainties were fully determined on standard concentration and peak areas. This provided the final expanded uncertainty on the unknown sample concentration.

Standards

The uncertainties for each standard concentration level ($u(Xc_i)$) are presented in Table A 15.

| Pesticides | $Xc_i \pm u(Xc_i)$ ($COV_i$ %)[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Target standard concentration level ($\mu g.L^{-1}$) | | | | | | | | | | | |
| | 0.05 | | 0.1 | | 0.5 | | 1 | | 2 | | 5 | |
| IPU | 0.0502 ± (6.2%) | 0.0031 | 0.1004 ± (3.5%) | 0.0035 | 0.502 ± (3.5%) | 0.018 | 1.004 ± (2.4%) | 0.025 | 2.009 ± (2.4%) | 0.050 | 5.02 ± (2.1%) | 0.11 |
| CTU | 0.0498 ± (6.2%) | 0.0030 | 0.0996 ± (3.5%) | 0.0035 | 0.498 ± (3.5%) | 0.018 | 0.996 ± (2.4%) | 0.025 | 1.993 ± (2.4%) | 0.049 | 4.98 ± (2.1%) | 0.11 |
| ATZ | 0.0502 ± (6.2%) | 0.0031 | 0.1005 ± (3.5%) | 0.0035 | 0.503 ± (3.5%) | 0.018 | 1.005 ± (2.4%) | 0.025 | 2.010 ± (2.4%) | 0.050 | 5.03 ± (2.1%) | 0.11 |
| CTL | 0.0513 ± (6.1%) | 0.0031 | 0.1026 ± (3.5%) | 0.0036 | 0.513 ± (3.5%) | 0.018 | 1.026 ± (2.4%) | 0.025 | 2.052 ± (2.4%) | 0.050 | 5.13 ± (2.1%) | 0.11 |
| PSC | 0.0603 ± (6.1%) | 0.0037 | 0.1206 ± (3.4%) | 0.0041 | 0.603 ± (3.4%) | 0.021 | 1.206 ± (2.2%) | 0.028 | 2.413 ± (2.2%) | 0.055 | 6.03 ± (1.9%) | 0.12 |
| FPP | 0.0619 ± (6.1%) | 0.0037 | 0.1238 ± (3.4%) | 0.0042 | 0.619 ± (3.4%) | 0.021 | 1.238 ± (2.2%) | 0.028 | 2.476 ± (2.2%) | 0.056 | 6.19 ± (1.9%) | 0.12 |
| ETF | 0.0509 ± (6.2%) | 0.0031 | 0.1018 ± (3.5%) | 0.0036 | 0.509 ± (3.5%) | 0.018 | 1.018 ± (2.4%) | 0.025 | 2.037 ± (2.4%) | 0.050 | 5.09 ± (2.1%) | 0.11 |
| MTL | 0.0626 ± (6.1%) | 0.0038 | 0.1252 ± (3.4%) | 0.0042 | 0.626 ± (3.4%) | 0.021 | 1.252 ± (2.2%) | 0.028 | 2.505 ± (2.2%) | 0.057 | 6.26 ± (1.9%) | 0.12 |
| MTZ | 0.0497 ± (6.2%) | 0.0030 | 0.0995 ± (3.5%) | 0.0035 | 0.498 ± (3.5%) | 0.018 | 0.995 ± (2.4%) | 0.025 | 1.990 ± (2.4%) | 0.049 | 4.98 ± (2.1%) | 0.11 |
| NPP | 0.0509 ± (6.2%) | 0.0031 | 0.1018 ± (3.5%) | 0.0036 | 0.509 ± (3.5%) | 0.018 | 1.019 ± (2.4%) | 0.025 | 2.037 ± (2.4%) | 0.050 | 5.09 ± (2.1%) | 0.11 |
| CYP | 0.0501 ± (6.2%) | 0.0031 | 0.1002 ± (3.5%) | 0.0035 | 0.501 ± (3.5%) | 0.018 | 1.003 ± (2.4%) | 0.025 | 2.005 ± (2.4%) | 0.050 | 5.01 ± (2.1%) | 0.11 |
| ACF | 0.0504 ± (6.2%) | 0.0031 | 0.1008 ± (3.5%) | 0.0035 | 0.504 ± (3.5%) | 0.018 | 1.008 ± (2.4%) | 0.025 | 2.016 ± (2.4%) | 0.050 | 5.040 ± (2.1%) | 0.108 |
| DFF | 0.0499 ± (6.2%) | 0.0031 | 0.0999 ± (3.5%) | 0.0035 | 0.500 ± (3.5%) | 0.018 | 0.999 ± (2.4%) | 0.025 | 2.00 ± (2.4%) | 0.050 | 5.00 ± (2.1%) | 0.11 |
| TBC | 0.0501 ± (6.2%) | 0.0031 | 0.1002 ± (3.5%) | 0.0035 | 0.501 ± (3.5%) | 0.018 | 1.002 ± (2.4%) | 0.025 | 2.005 ± (2.4%) | 0.050 | 5.01 ± (2.1%) | 0.11 |
| EPX | 0.0501 ± (6.2%) | 0.0031 | 0.1003 ± (3.5%) | 0.0035 | 0.502 ± (3.5%) | 0.018 | 1.003 ± (2.4%) | 0.025 | 2.006 ± (2.4%) | 0.050 | 5.02 ± (2.1%) | 0.11 |
| MFP | 0.0504 ± (6.2%) | 0.0031 | 0.1008 ± (3.5%) | 0.0035 | 0.504 ± (3.5%) | 0.018 | 1.008 ± (2.4%) | 0.025 | 2.016 ± (2.4%) | 0.050 | 5.04 ± (2.1%) | 0.11 |

**Table A 15: Standard concentrations, uncertainties, and associated coefficients of variation.[a] $Xc_i$: obtained standard concentration ($\mu g.L^{-1}$) for i = 1 to 6 levels after considering the actual weighed mass of each pesticide and each diluting step, $u(Xc_i)$: corresponding uncertainties, COVi (%) coefficient of variation on $Xc_i$.**

All coefficients of variations (COVs) were lower than 10% and decreased when the concentration levels were increased.

Model

Five replicates of each of the six concentration levels were analyzed. The peak areas were averaged over the five replicates for each concentration level. Standard concentrations of these six levels were determined by taking into account the mass of each pesticide that was actually measured and the subsequent dilution steps (Appendix, Eq. A.4). Table A 16 shows the model's coefficients ($b_1$ and $b_2$) and the associated uncertainties ($u(b_1)$ and $u(b_2)$) on the range in which the models were validated (Table A 16). High determination coefficients $r^2$ were obtained for each pesticide.

| | Calibration curve polynomials | | | | |
|---|---|---|---|---|---|
| Pesticide | $b_1$ | $b_2$ | $u(b_1)$ | $u(b_2)$ | $cov(b_1, b_2)$ |
| IPU | 560000 | -40000 | 14000 | 3000 | -3.89E+07 |
| CTU | 207600 | -12900 | 6100 | 1300 | -7.65E+06 |
| ATZ | 445200 | | 5600 | | |
| CTL | 308000 | 26100 | 18000 | 3900 | -6.85E+07 |
| PSC | 1200000 | | 18000 | | |
| FPP | 2580000 | 79800 | 170000 | 9100 | -1.53E+09 |
| ETF | 1309000 | | 8800 | | |
| MTL | 901100 | | 6300 | | |
| MTZ | 255700 | | 3400 | | |
| NPP | 4086000 | | 83000 | | |
| CYP | 473500 | | 7300 | | |
| ACF | 311000 | 121000 | 26000 | 14000 | -3.62E+08 |
| DFF | 3600000 | 410642 | 200000 | 42000 | -8.01E+09 |
| TBC | 869000 | 66000 | 52000 | 11000 | -5.70E+08 |
| EPX | 673000 | 270000 | 73000 | 40000 | -2.83E+09 |
| MFP | 1400000 | 190000 | 110000 | 23000 | -2.33E+09 |

**Table A 16: Calibration model coefficients, coefficient uncertainties, and covariances.**

Determination of real sample concentrations

As presented in Equations A.14 and A.20 (cf. Appendix), sample concentration uncertainty, stemming from the model, depends on the model's coefficient uncertainties $u(b_1)$ and $u(b_2)$, as previously shown (Table A 16) and the uncertainties on chromatogram peak areas (u(Ym)). Peak integration uncertainty was determined as a combination of repeatability, reproducibility, and software resolution. Chromatograms were all integrated manually, thus leading to possible differences in peak areas obtained depending on how the integrations were carried out. Good intra-analysis repeatability was noted with COVs varying between 0.3 and 7.9% and rarely exceeding 5%. Inter-analysis reproducibility COVs belonged to the 0.1–23.1% range. As for intra-analysis repeatability tests, the lowest concentrations were those affected by the highest variability. However, an exception was noted for cyproconazole, for which COVs were around 7% for all concentration levels. Because it was a two-enantiomer mixture, this pesticide presented two attached peaks whose tail slowly reached the baseline. The results showed a very low uncertainty due to software resolution, with COV rarely surpassing 2%. The weight of each of these three identified sources of variability on peak areas was calculated by dividing the variances (square of the standard deviation) by the square of the average area for each concentration level and pesticide compound. Among these three contributors to variability on peak areas, reproducibility among manipulators had the highest

weight on total peak area uncertainty. Because several analysts may use the method, it is important that the integration method be harmonized. The critical point is usually the end point an analyst selects on the tail of the chromatogram to determine its area.

Expanded uncertainties

The results presented in Table A 17 were obtained from chromatogram areas (Ym) from the analysis of samples presenting four different levels in the validated range of each pesticide.

| Pesticides | $X_i \pm u(Xm_i)$ $(COV_i\ \%)^{(a)}$ | | | |
|---|---|---|---|---|
| | Target standard concentration level ($\mu g.L^{-1}$) | | | |
| | level 1 | level 2 | level 3 | level 4 |
| IPU | 0.0522 ± 0.0046 (8.9%) | 0.224 ± 0.013 (5.6%) | 0.719 ± 0.031 (4.3%) | 1.297 ± 0.057 (4.3%) |
| CTU | 0.1178 ± 0.0083 (7.0%) | 0.695 ± 0.047 (6.8%) | 1.215 ± 0.061 (4.9%) | 2.08 ± 0.17 (8.0%) |
| ATZ | 0.1593 ± 0.0029 (1.8%) | 0.303 ± 0.009 (2.9%) | 0.594 ± 0.011 (1.8%) | 1.572 ± 0.022 (1.3%) |
| CTL | 0.703 ± 0.032 (4.5%) | 1.192 ± 0.045 (3.7%) | 2.102 ± 0.056 (2.6%) | 4.324 ± 0.045 (1.0%) |
| PSC | 0.0518 ± 0.0033 (6.3%) | 0.707 ± 0.013 (1.8%) | 1.443 ± 0.026 (1.8%) | 3.609 ± 0.072 (2.0%) |
| FPP | 0.0882 ± 0.0084 (9.5%) | 0.657 ± 0.044 (6.6%) | 1.417 ± 0.084 (5.9%) | 3.45 ± 0.16 (4.7%) |
| ETF | 0.0817 ± 0.0066 (8.1%) | 0.380 ± 0.012 (3.1%) | 1.565 ± 0.038 (2.4%) | 3.424 ± 0.076 (2.2%) |
| MTL | 0.1510 ± 0.0015 (1.0%) | 0.3104 ± 0.0045 (1.4%) | 1.556 ± 0.021 (1.3%) | 3.252 ± 0.043 (1.3%) |
| MTZ | 0.090 ± 0.011 (11.9%) | 0.326 ± 0.013 (4.0%) | 1.204 ± 0.021 (1.7%) | 2.432 ± 0.046 (1.8%) |
| NPP | 0.1147 ± 0.0046 (4.0%) | 0.2487 ± 0.0084 (3.4%) | 0.530 ± 0.017 (3.2%) | 1.292 ± 0.049 (3.7%) |
| CYP | 0.221 ± 0.020 (8.9%) | 0.477 ± 0.046 (9.6%) | 0.984 ± 0.068 (6.8%) | 2.10 ± 0.17 (8.1%) |
| ACF | 0.230 ± 0.017 (7.4%) | 0.481 ± 0.025 (5.2%) | 1.052 ± 0.049 (4.6%) | 1.728 ± 0.037 (2.1%) |
| DFF | 0.0595 ± 0.0035 (5.9%) | 0.309 ± 0.017 (5.5%) | 1.407 ± 0.051 (3.6%) | 3.199 ± 0.097 (3.0%) |
| TBC | 0.142 ± 0.012 (8.2%) | 0.568 ± 0.043 (7.5%) | 1.225 ± 0.051 (4.1%) | 2.646 ± 0.109 (4.1%) |
| EPX | 0.238 ± 0.022 (9.2%) | 0.495 ± 0.034 (6.8%) | 0.995 ± 0.046 (4.6%) | 1.426 ± 0.037 (2.6%) |
| MFP | 0.174 ± 0.012 (7.1%) | 0.414 ± 0.026 (6.3%) | 0.992 ± 0.047 (4.7%) | 2.602 ± 0.061 (2.3%) |

**Table A 17: Concentrations, uncertainties and associated coefficients of variation from the calibration modeling step.[(a)] $X_i$: concentration ($\mu g.L^{-1}$), $u(Xm_i)$: uncertainties on concentration caused by the model, COVi (%) coefficient of variation on $X_i$.**

In general, it should be noted that COVs were lower than 10%, except for the lowest level presented herein for MTZ (COV = 11.9% for 0.09 µg.$L^{-1}$) (Table A 17). It was observed that the highest COVs were associated with the smallest concentrations. This is due to decreased peak shape quality for samples whose concentrations were close to the LOQ, making the integrations of such chromatograms less repeatable (highest overall u(Ym)). In addition, MTL, ATZ, NPP, and CTL COVs were not over 5%. FPP, CTU, and CYP were the pesticides for which most of the concentration levels presented herein were associated with COVs belonging to a higher range (4.7–9.6%) than of the range found for the entire set of the 16 molecules (1–11.9%).

When fitting a calibration curve with the OLS method, uncertainties on pesticide standard concentrations are assumed to be zero. Consequently, when using this method, the final uncertainties on real sample concentrations may be underestimated. Other regression methods, such as those of Williamson [53] or York-Williamson [54, 55], consider that the uncertainties on both axes fit a model. However, this implies detailed uncertainty calculations. This is time-consuming and most software packages use the OLS method by default. It is, however, important to note that uncertainties depend on model coefficients derived from calibration quality. Nevertheless, even when trying to minimize uncertainties related to the entire calibration procedure as much as possible, those arising from instrument uncertainties and dilution steps will still be present. Therefore, the final uncertainty on a real sample concentration was calculated considering the uncertainty stemming from the standard preparation and the uncertainty caused by the model. Expanded uncertainties for different levels are provided in Table A 18.

As previously noted from individual uncertainties, the highest ones were associated with the lowest concentration levels. This is usually noted, as reported by Ratola et al. (2006) [56]. COVs ranged from 4.68% to 36.71%. The mean COV was 10.61%, whereas the median was 8.80%. Such uncertainties are fairly good compared to those found in the literature for similar analytical methods. Ratola et al. (2006) found uncertainties with values up to 50% but generally lower than 25% [56].

*Analysis of real samples*

This method was applied to 61 flow-weighted composite water samples taken at the outlet of a 46-ha subsurface artificially drained watershed [47] from April 2007 to July 2009. Blanks made of mineral water were first analyzed to confirm that no peak appeared at any of the 16 pesticide retention times. In addition, blanks were introduced every seventh sample and medium concentration level standards were analyzed at the end of a series to check peak retention times. Pesticides for which concentrations were higher than the LOQ (and frequency of quantification) were CTU (92%), MTZ (90%), IPU (85%), EPX (43%), and DFF (28%). Observed maximal peak concentrations and associated uncertainties from the previous calculations were 33.7 ± 0.2 (IPU), 13.2 ± 0.4 (CTU), 4.2 ± 0.2 (MTZ), 2.3 ± 0.1 (EPX), and 0.64 ± 0.05 (DFF) µg/L. As already noted [57, 58], pesticide concentrations were in accordance with farmer pesticide applications and rain flow events. Among the other pesticides, ATZ, CTL, PSC, FPP, ETF, CYP, and ACF were usually not detected and DFF, TBC, and MFP were on some occasions between the LODs and LOQs.

| Pesticides | $U(X_i)$ ($COV(X_i)$ %) | | | | | |
|---|---|---|---|---|---|---|
| | Average concentration level ($\mu g.L^{-1}$) | | | | | |
| | 0.05 | 0.1 | 0.5 | 1 | 2 | 5 |
| IPU | 0.011 (21.85%) | 0.012 (11.56%) | 0.044 (8.72%) | 0.066 (6.61%) | 0.12 (5.87%) | 0.24 (4.88%) |
| CTU | | 0.018 (18.01%) | 0.08 (16.01%) | 0.11 (10.66%) | 0.16 (7.80%) | 0.40 (7.93%) |
| ATZ | 0.0064 (12.80%) | 0.0074 (7.46%) | 0.036 (7.21%) | 0.053 (5.27%) | 0.10 (5.07%) | |
| CTL | | | 0.074 (14.74%) | 0.10 (10.27%) | 0.15 (7.54%) | 0.24 (4.71%) |
| PSC | 0.0099 (19.83%) | 0.0098 (9.81%) | 0.049 (9.70%) | 0.076 (7.63%) | 0.18 (9.08%) | 0.47 (9.43%) |
| FPP | 0.018 (36.71%) | 0.029 (29.04%) | 0.097 (19.37%) | 0.18 (17.74%) | 0.35 (17.40%) | 0.53 (10.52%) |
| ETF | | 0.015 (15.03%) | 0.043 (8.65%) | 0.061 (6.06%) | 0.13 (6.25%) | 0.27 (5.31%) |
| MTL | 0.0081 (16.34%) | 0.012 (12.31%) | 0.060 (12.04%) | 0.10 (10.24%) | 0.23 (11.41%) | 0.45 (9.09%) |
| MTZ | 0.016 (31.56%) | 0.023 (22.51%) | 0.044 (8.80%) | 0.058 (5.76%) | 0.11 (5.36%) | 0.23 (4.67%) |
| NPP | 0.0063 (12.63%) | 0.0075 (7.52%) | 0.037 (7.43%) | 0.053 (5.25%) | 0.11 (5.28%) | 0.238 (4.76%) |
| CYP | 0.0062 (12.40%) | 0.0070 (7.00%) | 0.053 (10.62%) | 0.10 (10.40%) | 0.17 (8.37%) | 0.40 (8.07%) |
| ACF | | 0.012 (12.37%) | 0.049 (9.84%) | 0.07 (7.02%) | 0.14 (6.95%) | |
| DFF | 0.0071 (14.33%) | 0.0098 (9.89%) | 0.05 (9.90%) | 0.084 (8.45%) | 0.14 (7.10%) | 0.29 (5.79%) |
| TBC | | 0.024 (24.23%) | 0.058 (11.57%) | 0.099 (9.90%) | 0.14 (7.12%) | 0.31 (6.14%) |
| EPX | | 0.014 (13.89%) | 0.056 (11.26%) | 0.084 (8.37%) | 0.13 (6.73%) | |
| MFP | 0.0065 (13.02%) | 0.0087 (8.72%) | 0.043 (8.67%) | 0.072 (7.20%) | 0.14 (6.86%) | 0.25 (4.96%) |

**Table A 18: Expanded uncertainties on real sample concentrations ($U(X_i)$) and associated coefficient of variations ($COV(X_i)$).**

**Conclusions**

This study showed that a single analytical method for the simultaneous analysis of 16 pesticides presenting a wide range of physicochemical characteristics was possible, provided one accepted compromises on the LOQs or specificity results obtained. The use of the DoE methodology helped develop the method with a limited number of experiments but considering a high (seven) number of factors. The LOQs between 0.05 and 0.5 µg/L found herein were acceptable for detecting and comparing results on water samples in agricultural areas, usually presenting high pesticide concentrations. In spite of slight limitations from the specificity results, the major validation steps of the analytical method were successful. It is particularly important for a result to be reliable to associate it with its uncertainty value. Consequently, the method was only considered fully validated after calculating fairly good expanded uncertainties on real sample concentrations that were mostly lower than 10%, rarely exceeded 20%, and were applied to real water samples.

**Acknowledgements:**

The authors wish to thank C. Margoum, P. Bados, C. Chaumont, and J. Brest for their help in the design of experiments, sample collection, or method validation procedures as well as B. Palagos for precious advice with R and statistical analyses.

**Appendix**:
Uncertainty calculations are detailed below.
(1) Six working standard solutions, whose target concentrations were 0.05, 0.1, 0.5, 1, 2, and 5 µg.L$^{-1}$, were prepared to obtain calibration curves according to the following steps:
1.1. Weighing of approximately 0.75 mg (herein noted m) of each pesticide (Sartorius ME5-F balance, supplier, city, country). The weighed mass value was carefully noted.
1.2. Introduction of the 16 pesticide masses into 15 mL (called Vm) of acetone SupraSolv (supplier, city, country) using a 10-mL pipette twice. An initial mother solution of approximately 50 mg.L$^{-1}$ (Cm) concentration for each molecule was thus obtained.
1.3. Preparation of the intermediate solution (Ci = 500 µg.L$^{-1}$) taking 180 µL (Vm') of the initial mother solution and diluting it into 18 mL (Vi): 10-mL and 100-µL pipettes were both used twice.
1.4. Six different dilutions of the intermediate solution were prepared in mineral water by taking Vi' mL of the intermediate solution to obtain the six standard working solutions, whose i = 1 to 6 levels, called Xci. Pipettes of 10-mL, 100-µL, 50-µL, and 10-µL volumes were used. The final standard solution volumes were noted as Vci.
When a value y is dependent on several parameters $x_1$, …xn, the general relationship linking the combined uncertainty $u^2(y)$ to the independent parameters that it depends on is derived from a Taylor series expansion. We will assume a first-order expansion for both linear and quadratic calibration models [59]:

$$u^2\left[y(x_1,...x_n)\right]=\sum_i\left(\frac{\partial y}{\partial x_i}\cdot u(x_i)\right)^2+2\cdot\sum_{i=1}^{N-1}\sum_{j=i+1}^{N}\frac{\partial y}{\partial x_i}\cdot\frac{\partial y}{\partial x_j}\cdot u(x_i,x_j)$$

**Eq.A 1**
Standards were obtained from a series of dilutions (steps 1.1. to 1.4.):

$$Xci=\frac{m\cdot V'i}{Vci\cdot Vi}$$

**Eq.A 2**

Considering the covariance as negligible among the four parameters, the corresponding u(Xci) uncertainty for each standard and pesticide concentration level i was calculated as follows:

$$u^2(Xci)=\left(\frac{\partial Xci}{\partial m}\cdot u(m)\right)^2+\left(\frac{\partial Xci}{\partial V'i}\cdot u(V'i)\right)^2+\left(\frac{\partial Xci}{\partial Vci}\cdot u(Vci)\right)^2+\left(\frac{\partial Xci}{\partial Vi}\cdot u(Vi)\right)^2$$

**Eq.A 3**

$$u^2(Xci)=\left(\frac{V'i}{Vci\cdot Vi}\cdot u(m)\right)^2+\left(\frac{m}{Vci\cdot Vi}\cdot u(V'i)\right)^2+\left(\frac{-m\cdot V'i}{Vi\cdot Vci^2}\cdot u(Vci)\right)^2+\left(\frac{-m\cdot V'i}{Vi^2Vci}\cdot u(Vi)\right)^2$$

**Eq.A 4**
(2) Calibration models (Y = f(X)) were subsequently obtained from i = 1 to 6 pesticide chromatogram peak areas ($y_{ic}$) and standard concentrations ($x_{ic}$). For seven of the 16 molecules, linear models described the relationship between $y_{ic}$ and $x_{ic}$, whereas second-order models were obtained for the other nine molecules.
The ordinary least squares (OLS) method [60, 61] was used to fit either linear or quadratic models f($b_1$, $b_2$, $x_{ic}$), written as follows:

$$Yc=b_1\cdot Xc$$

**Eq.A 5**
and

$$Yc=b_1\cdot Xc+b_2\cdot Xc^2$$

**Eq.A 6**

$u(b_1)$ and $u(b_2)$ variances and covariance terms were calculated under the OLS assumptions. The OLS method assumes that there is no uncertainty on $x_{ic}$, no bias on $y_{ic}$, the $y_{ic}$ uncertainties are all the same, and the $y_{ic}$ measures are not correlated.
The estimated variance-covariance matrix of the regression coefficients was:

$$\sigma^2 * ({}^tFF)^{-1}$$

**Eq.A 7**

where $\sigma^2$ is the error variance calculated as the sum of the square of the residuals divided by the number of observations ($n$) minus the number of parameters ($p$):

$$\sigma^2 = \frac{\sum [y_{ic} - f(b_1, b_2, x_{ic})]^2}{n - p}$$

**Eq.A 8**

F was the matrix containing the following terms:

$$F_{ij} = \frac{\partial f(\hat{\beta}, x_{ic})}{\partial \hat{\beta}_j}$$

**Eq.A 9**

$\hat{\beta}$ is a vector of parameters $b_1$ and/or $b_2$ for j =1 to p parameters.
Finally, ${}^tF$ is the transpose of matrix F.
For a linear model, $u(b_1)$ can be written as follows:

$$u(b_1) = \sigma^2 \frac{n}{n \cdot \sum_i x_{ic}^2 - \left(\sum_i x_{ic}\right)^2}$$

**Eq.A 10**

The R software [62] was used to derive model coefficients and associated variances and covariances according to the previous equations.
(3) Unknown concentrations (X) of real samples were then determined using reversed models $f^{-1}(b_1, b_2, y_{ic})$.
From linear calibration curves (Eq. A.5), the reversed model was written as follows:

$$X = \frac{Ym}{b_1}$$

**Eq.A 11**

The associated uncertainty caused by the model on the final concentration u(Xm) was determined using a Taylor development to the first order. For linear models, assuming no covariance between $b_1$ and Ym, it can be written as:

$$u^2(X) = \frac{1}{b_1^2} \cdot u(Ym)^2 + \frac{(Ym)^2}{b_1^2} \cdot \frac{u(b_1)^2}{b_1^2}$$

**Eq.A 12**

For second-order calibration models (Eq. A.6), X is one of the two roots of the solution of the following equation:

$$0 = b_2 \cdot X^2 + b_1 \cdot X - Ym$$

**Eq.A 13**

This equation discriminant $\Delta$ and the possible equation roots $X_1$ and $X_2$ are given below:

$$\Delta = b_1^2 - 4 \cdot b_2 \cdot (-Ym)$$

**Eq.A 14**

$$X_1 = \frac{-b_1 - \sqrt{\Delta}}{2 \cdot b_2}$$

**Eq.A 15**

$$X_2 = \frac{-b_1 + \sqrt{\Delta}}{2 \cdot b_2}$$

**Eq.A 16**

Root $X_2$ provided realistic results. Hence, for quadratic models, $X = X_2$. Let $f_q$ be the previous function (Eq. A.16.) linking X to $b_1$, $b_2$ and Ym.

$$u^2(X) = \left[\frac{\partial f_q}{\partial b_1}\right]^2 \cdot u(b_1)^2 + \left[\frac{\partial f_q}{\partial b_2}\right]^2 \cdot u(b_2)^2 + \left[\frac{\partial f_q}{\partial Ym}\right]^2 \cdot u(Ym)^2 + 2 \cdot \mathrm{cov}(b_1, b_2) \cdot \left[\frac{\partial f_q}{\partial b_1}\right] \cdot \left[\frac{\partial f_q}{\partial b_2}\right]$$

**Eq.A 17**

The corresponding developed equation is:

$$u^2(X) = \left[\frac{1}{2 \cdot b_2}\left(\frac{2 \cdot b_1 - 2\sqrt{b_1^2 + 4 \cdot b_2 \cdot Ym}}{2\sqrt{b_1^2 + 4 \cdot b_2 \cdot Ym}}\right)\right]^2 \cdot u(b_1)^2$$

$$+ \left[\frac{1}{2 \cdot b_2}\left(\frac{8 \cdot Ym \cdot b_2 + 4 \cdot b_1 \cdot \sqrt{b_1^2 + 4 \cdot b_2 \cdot Ym} - 4\left(b_1^2 + 4 \cdot b_2 \cdot Ym\right)}{4 \cdot b_2 \cdot \sqrt{b_1^2 + 4 \cdot b_2 \cdot Ym}}\right)\right]^2 \cdot u(b_2)^2$$

$$+ \left[\frac{4 \cdot b_2}{2 \cdot \sqrt{b_1^2 + 4 \cdot b_2 \cdot Ym}}\right]^2 \cdot u(Ym)^2$$

$$+ 2 \cdot \mathrm{cov}(b_1, b_2) \cdot \left[\frac{1}{2 \cdot b_2}\left(\frac{2 \cdot b_1 - 2 \cdot \sqrt{b_1^2 + 4 \cdot b_2 \cdot Ym}}{2 \cdot \sqrt{b_1^2 + 4 \cdot b_2 \cdot Ym}}\right)\right] \cdot \left[\frac{1}{2 \cdot b_2}\left(\frac{8 \cdot Ym \cdot b_2 + 4 \cdot b_1 \cdot \sqrt{b_1^2 + 4 \cdot b_2 \cdot Ym} - 4 \cdot \left(b_1^2 + 4 \cdot b_2 \cdot Ym\right)}{4 \cdot b_2 \cdot \sqrt{b_1^2 + 4 \cdot b_2 Ym}}\right)\right]$$

**Eq.A 18**

where Ym is the unknown sample chromatogram peak area, obtained using the SPME-GC/MS analytical method. Coefficients $b_1$ and $b_2$ are those obtained during the calibration (step 2). u(Ym) is the uncertainty on the chromatogram peak area calculated taking into account three identified sources of errors on peak chromatogram integration.

3.1. Intra-analyst repeatability uncertainty $u^2$(Ar_repeat)

One analyst integrated each of the 16 pesticide peaks ten times for each of the six standard concentration levels over a few days.

3.2. Inter-analyst reproducibility uncertainty $u^2$(Ar_repro)

Four different analysts integrated the six chromatograms of the standard solutions for the entire set of 16 molecules.

Variances around the mean of the ten values were used to estimate $u^2$(Ar_repeat) and $u^2$(Ar_repro).

3.3. Software resolution $u^2$(Areso)

X-Calibur software was used to obtain the values of chromatogram areas and had its own resolution. For each pesticide and each standard concentration, three integrations were carried out, providing three chromatogram peak area values. The first integration led to an area value called "$A_{center}$". The other two were obtained by moving the cursor from a single one step to the right ("$A_{right}$") and the left ("$A_{left}$"). The error was the difference between the center and the left or right areas. The highest error (either $A_{center}$-$A_{left}$ or $A_{center}$-$A_{right}$) was used to calculate the uncertainty by assuming a uniform distribution: $u^2(\mathrm{Areso}) = error / \sqrt{3}$.

$$u(Ym) = \sqrt{u^2(Ar\_repro) + u^2(Ar\_repeat) + u^2(Areso)}$$

**Eq.A 19**

(4) Considering that the OLS assumption does not account for uncertainty on standard concentration except in the $b_1$ and $b_2$ coefficients, they should also be added in the final uncertainty on the real sample concentration.

The combined uncertainties on the real sample concentrations u(X) were calculated as follows:

$$u(X) = \sqrt{u^2(Xc) + u^2(Xm)}$$

**Eq.A 20**

The final expanded uncertainties (U(X)) on the real sample concentrations were determined considering a coverage factor of 2 for a 95% level of confidence:

$$U(X) = 2 \cdot u(X)$$

**Eq.A 21**

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## Appendix V Gas phase composition in experiments without EPX

On day 160 after the start of the incubations, gas samples from the five unlabelled systems were taken by means of Catener void tubes and gas syringes. Previous opening of the unlabelled incubations was approximately 2 weeks before. A 2-week period between each times the systems were open to change NaOH vials was common. The gas samples would therefore approximately represent the gas composition one can expect at this stage of the experiment. We aimed at understanding how reduced the systems were by analyzing their major gaseous components. Gas samples were analyzed for their composition including $O_2$, $N_2$, $N_2O$, $NH_3$, $CO_2$, $CH_4$ and $H_2S$, by means of a gas chromatography (micro-gc CP-4900 Quad, Varian, and CPMAITRE ELITE software 3.2, Les Ulis, France). The results showed that $N_2O$, $NH_3$ and $H_2S$ were not detected. However, the systems were slightly anoxic but methane production was low compared to $CO_2$ production. Oxygen concentrations (20–21 %) were fairly close to air oxygen concentration. This value was not very accurate because the gas chromatography apparatus was calibrated with air and not with pure oxygen. However, it shows that the air overlaying the water/sediments systems had a composition close to air composition but included some traces of anoxic processes. The proportion of $CO_2$ obtained from anoxic and aerobic degradation processes of the initial organic carbon was estimated. For that, we considered that methanogenesis would lead to a 60% $CH_4$ and 40% $CO_2$ composition. Under this assumption, we calculated that the proportion of total $CO_2$ due to anoxic processes accounted for 14 to 24 %, whereas that produced by aerobic degradation was between 76 and 86 % (Table xx). Consequently, methanogenesis was not the major reaction pathway, despite the presence of reducing conditions. However, some degradation of the initial organic matter into methane can not be excluded.

| | **Gas composition compared to initial carbon mass (%)** | | **$CO_2$ portion attributed to aerobic/anoxic reaction pathways (%)** | |
|---|---|---|---|---|
| | **%C-$CO_2$** | **%C-$CH_4$** | **anoxic** | **aerobic** |
| **SB** | 37.6 | 13.6 | 24 | 76 |
| **SG** | 27.3 | 8.8 | 22 | 78 |
| **SF** | 15.7 | 4.2 | 18 | 82 |
| **P** | 11.4 | 3.0 | 17 | 83 |
| **F** | 9.3 | 1.9 | 14 | 86 |

**Table A 19: Gas composition and $CO_2$ metabolism origin estimations from incubations without EPX.**

## Appendix VI Incubations with $^{14}$C-Epoxiconazole – Supplemental information

| Substrate | Time | Mineralization | | | Water extractable | | | Methanol extractable | | | NER | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | d | % of initial $^{14}$C-EPX | | | | | | | | | | | | | | |
| SB | 0 | 0.0 | ± | 0.0 | 2.2 | ± | 0.2 | 97.0 | ± | 0.4 | 2.8 | ± | 0.9 | 102.1 | ± | 0.7 |
| | 14 | 0.2 | ± | 0.0 | 1.7 | ± | 0.1 | 91.4 | ± | 3.6 | 9.5 | ± | 2.4 | 102.6 | ± | 5.8 |
| | 77 | 0.6 | ± | 0.0 | 1.2 | ± | 0.1 | 76.1 | ± | 0.9 | 22.4 | ± | 3.5 | 100.3 | ± | 4.2 |
| | 177 | 1.1 | ± | 0.1 | 1.0 | ± | 0.0 | 76.2 | ± | 0.2 | 17.4 | ± | 1.6 | 95.8 | ± | 1.5 |
| SG | 0 | 0.0 | ± | 0.0 | 3.4 | ± | 0.1 | 101.6 | ± | 1.1 | 3.4 | ± | 0.5 | 108.5 | ± | 1.3 |
| | 14 | 0.1 | ± | 0.0 | 2.4 | ± | 0.1 | 100.9 | ± | 2.2 | 6.4 | ± | 1.2 | 109.8 | ± | 3.2 |
| | 77 | 1.2 | ± | 0.1 | 1.6 | ± | 0.0 | 87.0 | ± | 4.0 | 15.5 | ± | 1.0 | 105.4 | ± | 4.7 |
| | 177 | 3.9 | ± | 0.4 | 1.6 | ± | 0.1 | 78.3 | ± | 2.9 | 17.4 | ± | 1.6 | 101.2 | ± | 3.9 |
| SF | 0 | 0.0 | ± | 0.0 | 1.6 | ± | 0.1 | 99.8 | ± | 0.8 | 2.2 | ± | 0.1 | 103.7 | ± | 0.9 |
| | 14 | 0.2 | ± | 0.0 | 1.6 | ± | 0.1 | 97.8 | ± | 2.4 | 6.8 | ± | 0.2 | 106.2 | ± | 2.4 |
| | 77 | 0.9 | ± | 0.0 | 1.2 | ± | 0.0 | 98.1 | ± | 1.0 | 14.7 | ± | 0.7 | 114.8 | ± | 1.7 |
| | 177 | 2.7 | ± | 0.1 | 1.2 | ± | 0.1 | 87.7 | ± | 1.1 | 16.9 | ± | 0.2 | 108.5 | ± | 0.8 |
| P | 0 | 0.0 | ± | 0.0 | 16.2 | ± | 0.8 | 87.6 | ± | 1.8 | 2.9 | ± | 0.1 | 106.7 | ± | 1.1 |
| | 14 | 0.0 | ± | 0.0 | 16.8 | ± | 1.8 | 85.4 | ± | 1.4 | 3.7 | ± | 0.1 | 105.9 | ± | 2.8 |
| | 77 | 0.3 | ± | 0.0 | 13.3 | ± | 0.4 | 80.2 | ± | 0.6 | 9.5 | ± | 0.6 | 103.2 | ± | 0.9 |
| | 177 | 1.5 | ± | 0.4 | 18.8 | ± | 1.8 | 76.2 | ± | 6.5 | 29.8 | ± | 6.6 | 126.3 | ± | 4.4 |
| F | 0 | 0.0 | ± | 0.0 | 5.9 | ± | 0.6 | 99.3 | ± | 0.9 | 1.1 | ± | 0.0 | 106.3 | ± | 0.4 |
| | 14 | 0.1 | ± | 0.0 | 5.2 | ± | 0.1 | 99.6 | ± | 1.0 | 3.4 | ± | 0.1 | 108.2 | ± | 1.1 |
| | 77 | 0.5 | ± | 0.0 | 4.2 | ± | 0.7 | 93.9 | ± | 0.3 | 9.6 | ± | 0.4 | 108.2 | ± | 1.0 |
| | 177 | 2.0 | ± | 0.0 | 7.3 | ± | 0.6 | 83.8 | ± | 2.0 | 16.3 | ± | 0.3 | 109.4 | ± | 1.9 |

**Table A 20: Radioactivity distribution as percentage of initial $^{14}$C-EPX applied. [(a)]NER are non-extractable residues. Results are presented as average ± standard error over three replicates.**

| Substrate | Time | Water and methanol extracts composition | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 21.5 min | | | EPX 24.5 min | | | 24.8 min | | | Other molecules | | |
| | d | % of initial $^{14}$C-EPX | | | | | | | | | | | |
| SB | 0 | 0.0 | ± | 0.0 | 98.3 | ± | 0.7 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| | 14 | 0.0 | ± | 0.0 | 91.0 | ± | 2.5 | 2.0 | ± | 2.0 | 0.0 | ± | 0.0 |
| | 77 | 2.5 | ± | 2.2 | 49.2 | ± | 2.7 | 25.2 | ± | 2.0 | 0.0 | ± | 0.0 |
| | 177 | 2.5 | ± | 1.3 | 28.3 | ± | 2.8 | 40.3 | ± | 3.5 | 5.1 | ± | 1.4 |
| SG | 0 | 0.0 | ± | 0.0 | 105.0 | ± | 1.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| | 14 | 0.0 | ± | 0.0 | 81.8 | ± | 2.2 | 20.7 | ± | 1.0 | 0.8 | ± | 0.7 |
| | 77 | 3.7 | ± | 0.4 | 24.7 | ± | 6.7 | 60.0 | ± | 2.8 | 0.2 | ± | 0.1 |
| | 177 | 0.0 | ± | 0.0 | 9.9 | ± | 1.1 | 59.6 | ± | 2.6 | 9.0 | ± | 0.3 |
| SF | 0 | 0.0 | ± | 0.0 | 101.0 | ± | 0.6 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| | 14 | 0.0 | ± | 0.0 | 97.5 | ± | 2.2 | 0.0 | ± | 0.0 | 0.1 | ± | 0.1 |
| | 77 | 0.0 | ± | 0.0 | 95.7 | ± | 2.2 | 3.3 | ± | 3.3 | 0.0 | ± | 0.0 |
| | 177 | 0.8 | ± | 0.8 | 64.1 | ± | 4.6 | 21.4 | ± | 2.1 | 0.1 | ± | 0.1 |
| P | 0 | 1.6 | ± | 1.6 | 97.1 | ± | 5.6 | 0.0 | ± | 0.0 | 5.7 | ± | 2.9 |
| | 14 | 0.4 | ± | 0.2 | 100.2 | ± | 2.3 | 0.0 | ± | 0.0 | 0.5 | ± | 0.2 |
| | 77 | 27.9 | ± | 3.4 | 62.0 | ± | 4.3 | 0.1 | ± | 0.1 | 2.1 | ± | 0.8 |
| | 177 | 40.0 | ± | 8.3 | 35.7 | ± | 3.6 | 15.3 | ± | 4.8 | 2.4 | ± | 1.1 |
| F | 0 | 0.0 | ± | 0.0 | 104.6 | ± | 1.0 | 0.7 | ± | 0.7 | 0.0 | ± | 0.0 |
| | 14 | 0.0 | ± | 0.0 | 103.8 | ± | 1.9 | 0.0 | ± | 0.0 | 0.7 | ± | 0.7 |
| | 77 | 1.3 | ± | 1.6 | 92.7 | ± | 1.7 | 0.0 | ± | 0.0 | 0.3 | ± | 0.2 |
| | 177 | 3.0 | ± | 1.3 | 76.8 | ± | 5.3 | 3.2 | ± | 3.2 | 0.1 | ± | 0.1 |

**Table A 21: Water and methanol extractable fractions composition as percentage of initial $^{14}$C-EPX applied. Results are presented as average ± standard error over three replicates.**

**Appendix VII** **Buffer zone peak flow rates**

| n | Date inlet peak | Peak in L/s | Date outlet peak | Peak out L/s | Peak in-out L/s | Peak ratio out/in | Peak reduc % | $T_n$ (h) |
|---|---|---|---|---|---|---|---|---|
| 1 | 27/05/2008 18:45 | 44.8 | 28/5/08 6:15 | 36.690 | 8.070 | 0.820 | 82.0 | 2.0 |
| 2 | 06/02/2010 12:30 | 36.1 | 6/2/10 16:00 | 38.540 | -2.480 | 1.069 | 106.9 | 2.5 |
| 3 | 31/05/2008 19:00 | 35.5 | 31/5/08 18:00 | 28.970 | 6.560 | 0.815 | 81.5 | 2.6 |
| 4 | 21/04/2008 10:15 | 33.3 | 21/4/08 11:45 | 32.840 | 0.450 | 0.986 | 98.6 | 2.8 |
| 5 | 06/02/2010 05:15 | 33.1 | 6/2/10 5:45 | 25.510 | 7.600 | 0.770 | 77.0 | 2.8 |
| 6 | 29/12/2009 09:15 | 32.3 | 29/12/09 9:15 | 24.790 | 7.540 | 0.767 | 76.7 | 2.8 |
| 7 | 25/02/2010 20:45 | 29.6 | 25/2/10 21:30 | 24.820 | 4.740 | 0.840 | 84.0 | 3.1 |
| 8 | 28/12/2009 21:45 | 28.6 | 28/12/09 0:15 | 22.570 | 6.040 | 0.789 | 78.9 | 3.2 |
| 9 | 16/01/2010 18:00 | 27.6 | 16/01/2010 18:30 | 21.36 | 6.200 | 0.775 | 77.5 | 3.3 |
| 10 | 02/03/2010 07:15 | 26.7 | 2/3/10 8:15 | 23.660 | 3.010 | 0.887 | 88.7 | 3.4 |
| 11 | 25/02/2010 13:30 | 26.3 | 25/2/10 14:15 | 23.730 | 2.600 | 0.901 | 90.1 | 3.5 |
| 12 | 30/12/2009 10:15 | 25.7 | 30/12/09 11:30 | 22.250 | 3.420 | 0.867 | 86.7 | 3.6 |
| 13 | 11/05/2009 22:30 | 25.3 | 11/5/09 23:15 | 22.810 | 2.470 | 0.902 | 90.2 | 3.6 |
| 14 | 21/12/2009 21:30 | 22.6 | 22/12/09 3:30 | 16.740 | 5.820 | 0.742 | 74.2 | 4.1 |
| 15 | 01/01/2010 06:45 | 22.5 | 1/1/10 8:30 | 20.760 | 1.740 | 0.923 | 92.3 | 4.1 |
| 16 | 17/05/2009 06:45 | 22.2 | 17/5/09 8:00 | 19.850 | 2.320 | 0.895 | 89.5 | 4.1 |
| 17 | 29/01/2010 19:45 | 22.0 | 29/1/10 21:15 | 21.070 | 0.930 | 0.958 | 95.8 | 4.2 |
| 18 | 24/02/2010 15:30 | 21.0 | 24/2/10 16:30 | 20.870 | 0.130 | 0.994 | 99.4 | 4.4 |
| 19 | 30/01/2010 16:45 | 18.1 | 30/1/10 17:45 | 19.16 | -1.050 | 1.058 | 105.8 | 5.1 |
| 20 | 03/04/2010 20:45 | 17.0 | 3/4/10 20:45 | 17.470 | -0.470 | 1.028 | 102.8 | 5.4 |
| 21 | 13/01/2010 15:30 | 14.4 | 13/1/10 22:15 | 4.990 | 9.450 | 0.346 | 34.6 | 6.3 |
| 22 | 08/12/2007 19:00 | 13.7 | 8/12/07 19:00 | 7.380 | 6.290 | 0.540 | 54.0 | 6.7 |
| 23 | 05/02/2010 05:30 | 13.6 | 5/2/10 6:30 | 14.920 | -1.310 | 1.096 | 109.6 | 6.7 |
| 24 | 28/02/2010 02:00 | 12.7 | 28/2/10 3:00 | 15.550 | -2.880 | 1.227 | 122.7 | 7.2 |
| 25 | 23/11/2007 15:00 | 10.9 | 23/11/07 12:15 | 6.370 | 4.533 | 0.584 | 58.4 | 8.4 |
| 26 | 21/11/2007 05:30 | 10.8 | 21/11/07 8:15 | 8.240 | 2.548 | 0.764 | 76.4 | 8.5 |
| 27 | 23/02/2010 16:00 | 9.6 | 23/2/10 17:45 | 10.610 | -1.000 | 1.104 | 110.4 | 9.5 |
| 28 | 09/11/2008 21:00 | 9.4 | 10/11/2008 14:00 | 1.7 | 7.690 | 0.181 | 18.1 | 9.8 |
| 29 | 30/03/2010 03:15 | 8.9 | 30/03/2010 12:30 | 1.180 | 7.760 | 0.132 | 13.2 | 10.3 |
| 30 | 20/01/2010 15:45 | 8.4 | 20/01/2010 17:15 | 5.8 | 2.590 | 0.691 | 69.1 | 10.9 |
| 31 | 29/12/2007 19:30 | 8.3 | 30/12/07 6:45 | 2.790 | 5.470 | 0.338 | 33.8 | 11.1 |
| 32 | 30/04/2008 02:30 | 7.9 | 30/4/08 4:00 | 4.970 | 2.940 | 0.628 | 62.8 | 11.6 |
| 33 | 28/03/2010 09:45 | 7.7 |  | 0.000 | 7.720 | 0.000 | 0.0 | 11.9 |
| 34 | 16/01/2010 05:30 | 7.3 | 16/01/2010 06:00 | 4.41 | 2.870 | 0.606 | 60.6 | 12.6 |
| 35 | 25/05/2009 22:45 | 7.1 | 26/5/09 1:45 | 3.130 | 3.960 | 0.441 | 44.1 | 12.9 |
| 36 | 14/01/2010 20:00 | 7.0 | 14/01/2010 22:45 | 3.97 | 3.030 | 0.567 | 56.7 | 13.1 |
| 37 | 10/03/2009 12:15 | 6.9 | 10/3/09 15:00 | 1.490 | 5.400 | 0.216 | 21.6 | 13.3 |
| 38 | 22/02/2010 03:30 | 6.3 | 22/2/10 4:00 | 8.020 | -1.740 | 1.277 | 127.7 | 14.6 |
| 39 | 30/03/2010 16:15 | 6.2 | 30/3/10 20:00 | 2.290 | 3.880 | 0.371 | 37.1 | 14.9 |
| 40 | 15/12/2008 02:00 | 5.7 | 15/12/2008 04:00 | 5.99 | -0.270 | 1.047 | 104.7 | 16.0 |
| 41 | 04/02/2010 02:30 | 5.0 | 4/2/10 11:00 | 3.620 | 1.380 | 0.724 | 72.4 | 18.3 |
| 42 | 24/11/2008 05:45 | 4.5 | 24/11/2008 06:15 | 0.5 | 4.000 | 0.111 | 11.1 | 20.4 |
| 43 | 25/01/2010 08:00 | 4.4 | 25/01/2010 10:15 | 2.61 | 1.780 | 0.595 | 59.5 | 20.9 |
| 44 | 04/05/2009 05:00 | 4.1 | 14/5/09 8:30 | 3.110 | 1.000 | 0.757 | 75.7 | 22.3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 45 | 08/03/2009 17:30 | 3.8 | 08/03/2009 20:30 | 1.93 | 1.850 | 0.511 | 51.1 | 24.3 |
| 46 | 01/11/2008 06:45 | 3.5 | 1/11/08 9:30 | 1.170 | 2.330 | 0.334 | 33.4 | 26.2 |
| 47 | 04/03/2009 14:00 | 3.4 | 04/03/2009 16:00 | 2.35 | 1.090 | 0.683 | 68.3 | 26.6 |
| 48 | 27/10/2008 09:00 | 3.2 | 27/10/08 11:30 | 1.450 | 1.720 | 0.457 | 45.7 | 28.9 |
| 49 | 30/10/2008 10:30 | 3.0 | 30/10/08 12:30 | 0.750 | 2.250 | 0.250 | 25.0 | 30.6 |
| 50 | 26/03/2010 10:00 | 3.0 | | 0.000 | 3.000 | 0.000 | 0.0 | 30.6 |
| 51 | 21/05/2009 11:00 | 2.8 | 21/5/09 14:30 | 1.540 | 1.290 | 0.544 | 54.4 | 32.4 |
| 52 | 10/03/2009 19:00 | 2.7 | 10/3/09 23:00 | 2.06 | 0.610 | 0.772 | 77.2 | 34.3 |
| 53 | 10/05/2010 21:45 | 2.5 | 10/05/2010 21:45 | 0.000 | 2.500 | 0.000 | 0.0 | 36.7 |
| 54 | 16/10/2008 06:00 | 2.2 | 16/10/08 7:45 | 0.314 | 1.906 | 0.141 | 14.1 | 41.3 |
| 55 | 06/11/2008 02:45 | 1.9 | 6/11/08 5:00 | 0.670 | 1.220 | 0.354 | 35.4 | 48.5 |
| 56 | 02/11/2008 21:45 | 1.8 | 3/11/08 1:00 | 0.590 | 1.190 | 0.331 | 33.1 | 51.5 |

**Table A 22: Artificial wetland inlet and outlet peak flow rates and ratio.**

| n | Date inlet peak | Peak in (L/s) | Date outlet peak | Peak out (L/s) | in-out peak (L/s) | Peak ratio out/in (-) | Peak reduction (%) |
|---|---|---|---|---|---|---|---|
| 1 | 04/12/2008 16:15 | 19.2 | 4/12/08 16:15 | 9.7 | 9.5 | 0.50 | 50.4 |
| 2 | 05/01/2008 08:00 | 24.8 | 5/1/08 8:45 | 9.5 | 15.3 | 0.38 | 38.3 |
| 3 | 05/01/2008 22:15 | 24.8 | 5/1/08 21:15 | 9.3 | 15.5 | 0.38 | 37.5 |
| 4 | 08/12/2007 18:30 | 15.6 | 8/12/07 19:00 | 9.0 | 6.6 | 0.58 | 57.7 |
| 5 | 06/02/2010 05:15 | 11.1 | 6/2/10 7:15 | 7.2 | 3.9 | 0.65 | 64.8 |
| 6 | 29/12/2007 19:30 | 9.2 | 29/12/07 22:00 | 6.8 | 2.4 | 0.74 | 74.1 |
| 7 | 03/01/2008 23:15 | 7.0 | 4/1/08 1:45 | 6.6 | 0.4 | 0.94 | 94.3 |
| 8 | 09/11/2008 21:45 | 7.4 | 9/11/08 22:30 | 5.5 | 2.0 | 0.74 | 73.5 |
| 9 | 03/01/2008 05:45 | 5.5 | 3/1/08 7:45 | 5.1 | 0.4 | 0.92 | 92.2 |
| 10 | 29/12/2009 09:00 | 10.4 | 29/12/09 12:15 | 3.4 | 7.0 | 0.33 | 33.0 |
| 11 | 21/01/2009 16:00 | 8.0 | 21/1/09 16:30 | 3.0 | 5.0 | 0.37 | 37.1 |
| 12 | 25/02/2010 21:00 | 4.8 | 25/2/10 22:45 | 2.8 | 2.0 | 0.58 | 57.5 |
| 13 | 15/12/2008 02:45 | 2.9 | 15/12/08 2:30 | 2.0 | 0.8 | 0.71 | 70.5 |
| 14 | 27/01/2009 18:15 | 1.7 | 27/1/9 19.45 | 1.2 | 0.5 | 0.71 | 71.4 |

**Table A 23: Forest buffer inlet and outlet peak flow rates and ratio.**

**Appendix VIII** **Mass balances for water volumes, suspended sediments and nitrate.**

*Foreword*

Some negative load reductions were calculated. They were systematically associated with inlet and / or outlet concentrations below the limit of quantification. In addition, these results mostly came from samples previously suspected to present overestimated values but for which any analytical problem was clearly identified. For instance, cyproconazole was not detected at the wetland inlet but a concentration < LQ was observed at the outlet on 3 and 18 June 2008. The same occurred for aclonifen on 3 June 2008 and cyproconazole on 12 Dec. 2007. In 2008 – 2009, one fenpropidin inlet concentration was lower than the LQ whereas a 0.074 ± 0.02 µg/L outlet concentration was recorded (05 March 2009). The -127 % load reduction recorded for ethofumesate in 2009 – 2010 was only due to one sample pair collected on 11 May 2010 for which the molecule was not detected at the inlet and was <LQ at the outlet. In addition, during the sampling period, very low volumes passed through the inlet as it was the end of the 2009 – 2010 hydrologic year. Outlet volumes were larger indicating that the wetland was emptying out. The same situation explains tebuconazole and mefenpyr-diethyl negative load reductions. Errors therefore affected mass balance estimations in such cases. Unreliable load calculations were apparent similarly as for concentration reduction estimations. Apart from tebuconazole, molecules exhibiting negative load reductions in some occasions were generally those for which the number of inlet and outlet concentration pairs meeting our criteria was low.

| Molecule | 2007 - 2008 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AWin | | AWout | | Annual reduction in AW | FBin | | FBout | | Annual reduction in FB | Ditch | WSout | Stream | Reduction[(d)] | |
| | load | AWin/ WSout (%) | load | AWout/ AWin (%) | % | load | FBin/ WSout (%) | load | FBout/ FBin (%) | % | load | load | load | load | %WSout |
| Water volumes[(a)] | 44224 | 41 | 17425 | 39 | 61 | 11651 | 11 | 8756 | 75 | 25 | 53317 | 109192 | 79498 | 29694 | 27 |
| Suspended sediments[(b)] | 7358 | 38 | 1696 | 23 | 77 | 1527 | 8 | 596 | 39 | 61 | 10670 | 19555 | 12963 | 6592 | 34 |
| Nitrate[(b)] | 1553 | 32 | 553 | 36 | 64 | 750 | 16 | 547 | 73 | 27 | 2498 | 4801 | 3598 | 1202 | 25 |
| Isoproturon[(c)] | 75714 | 40 | 52135 | 36 | 64 | 42612 | 23 | 33865 | 79 | 21 | 70383 | 188709 | 156384 | 32325 | 17 |
| Chlorotoluron[(c)] | 77368 | 44 | 21261 | 27 | 73 | 20163 | 11 | 8133 | 40 | 60 | 77854 | 175384 | 107248 | 68136 | 39 |
| Atrazine[(c)] | 364 | 19 | 8 | 28 | 72 | 2 | 0 | 0 | 0 | 100 | 1519 | 1885 | 1527 | 358 | 19 |
| Chlorothalonil[(c)] | 2028 | 64 | 769 | 38 | 62 | 45 | 1 | 0 | 0 | 100 | 1089 | 3162 | 1858 | 1304 | 41 |
| Prosulfocarb[(c)] | 2246 | 25 | 5 | 0 | 100 | 2022 | 22 | 0 | 0 | 100 | 4836 | 9105 | 4842 | 4263 | 47 |
| Fenpropidine[(c)] | 163 | 42 | 62 | 38 | 62 | 33 | 12 | 25 | 77 | 23 | 87 | 283 | 174 | 109 | 39 |
| Ethofumesate[(c)] | 4458 | 42 | 105 | 2 | 98 | 132 | 1 | 0 | 0 | 100 | 6120 | 10709 | 6224 | 4485 | 42 |
| S-metolachlor[(c)] | 268 | 59 | 79 | 30 | 70 | 5 | 1 | 0 | 0 | 100 | 183 | 456 | 263 | 193 | 42 |
| Metazachlor[(c)] | 97430 | 41 | 29321 | 30 | 70 | 24384 | 10 | 17811 | 73 | 27 | 116734 | 238548 | 163866 | 74683 | 31 |
| Napropamide[(c)] | 394 | 41 | 80 | 20 | 80 | 115 | 12 | 79 | 68 | 32 | 456 | 965 | 614 | 351 | 36 |
| Cyproconazole[(c)] | 266 | 55 | 654 | 246 | -146 | 73 | 15 | 253 | 348 | -248 | 146 | 484 | 1053 | -569 | -118 |
| Aclonifen[(c)] | 24 | 17 | 55 | 229 | -129 | 0 | 0 | 0 | 0 | 100 | 119 | 144 | 175 | -31 | -22 |
| Diflufenican[(c)] | 2318 | 61 | 339 | 15 | 85 | 287 | 8 | 79 | 28 | 72 | 1211 | 3816 | 1629 | 2188 | 57 |
| Tebuconazole[(c)] | 4875 | 34 | 408 | 8 | 92 | 2079 | 14 | 578 | 28 | 72 | 7546 | 14500 | 8532 | 5968 | 41 |
| Mefenpyr-dietyl[(c)] | 2805 | 17 | 38 | 1 | 99 | 55 | 0 | 0 | 0 | 100 | 13231 | 16091 | 13269 | 2822 | 18 |
| Epoxiconazole[(c)] | 9315 | 69 | 3426 | 37 | 63 | 171 | 1 | 51 | 30 | 70 | 3958 | 13444 | 7435 | 6010 | 45 |
| Mean (pesticides) | | 42 | | 49 | 51 | | 8 | | 48 | 52 | | | | | 23 |
| Median (pesticides) | | 41 | | 29 | 71 | | 9 | | 28 | 72 | | | | | 39 |

**Table A 24: 2007 – 2008: Artificial wetland (AW) and forest buffer (FB) mass balances for the sixteen pesticides belonging to the analytical method. WSout, in and out stand for "watershed outlet", "inlet" and "outlet", respectively. [(a)] Water volumes are given in $m^3$. [(b)]Suspended sediments and nitrate units are kg. [(c)]Pesticide loads are in mg. [(d)]Reduction corresponded to the portion of pesticides that was actually dissipated through the two buffer zones and did not reach the stream. In italic are values for which problem in the analytical procedures were detected.**

| Molecule | 2008 - 2009 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AWin | | AWout | | Annual reduction in AW | FBin | | FBout | | Annual reduction in FB | Ditch | WSout | Stream | Reduction[(d)] | |
| | load | AWin/ WSout (%) | load | AWout/ AWin (%) | % | load | FBin/ WSout (%) | load | FBout/ FBin (%) | % | load | load | load | load | %WSout |
| Water volumes[(a)] | 19256 | 31 | 8178 | 42 | 58 | 8129 | 13 | 5855 | 72 | 28 | 35667 | 63052 | 49700 | 13352 | 21 |
| Suspended sediments[(b)] | 2109 | 14 | 235 | 11 | 89 | 1106 | 7 | 730 | 66 | 34 | 12281 | 15496 | 13246 | 2250 | 15 |
| Nitrate[(b)] | 759 | 35 | 182 | 24 | 76 | 373 | 17 | 203 | 54 | 46 | 1046 | 2178 | 1431 | 747 | 34 |
| Isoproturon[(c)] | 95462 | 10 | 55957 | 59 | 41 | 15804 | 2 | 206 | 1 | 99 | 870210 | 981476 | 926373 | 55102 | 6 |
| Chlorotoluron[(c)] | 11099 | 24 | 3777 | 34 | 66 | 2856 | 6 | 80 | 3 | 97 | 32986 | 46941 | 36843 | 10098 | 22 |
| Atrazine[(c)] | 103 | 50 | 29 | 28 | 72 | 37 | 18 | 22 | 59 | 41 | 66 | 206 | 117 | 89 | 43 |
| Chlorothalonil[(c)] | 989 | 22 | 239 | 24 | 76 | 456 | 10 | 264 | 58 | 42 | 2967 | 4411 | 3470 | 942 | 21 |
| Prosulfocarb[(c)] | 105 | 95 | 12 | 12 | 88 | 1 | 1 | 0 | 0 | 100 | 5 | 111 | 17 | 93 | 84 |
| Fenpropidine[(c)] | 224 | 39 | 48 | 21 | 79 | 41 | 7 | 44 | 109 | -9 | 303 | 567 | 395 | 172 | 30 |
| Ethofumesate[(c)] | 324 | 45 | 40 | 12 | 88 | 81 | 11 | 42 | 52 | 48 | 312 | 718 | 394 | 324 | 45 |
| S-metolachlor[(c)] | 209 | 61 | 16 | 8 | 92 | 73 | 21 | 18 | 25 | 75 | 62 | 344 | 96 | 248 | 72 |
| Metazachlor[(c)] | 8558 | 26 | 2618 | 31 | 69 | 4214 | 13 | 1277 | 30 | 70 | 20179 | 32951 | 24074 | 8878 | 27 |
| Napropamide[(c)] | 201 | 45 | 29 | 15 | 85 | 185 | 41 | 40 | 22 | 78 | 60 | 446 | 129 | 317 | 71 |
| Cyproconazole[(c)] | 580 | 20 | 251 | 43 | 57 | 51 | 2 | 35 | 69 | 31 | 2294 | 2925 | 2580 | 345 | 12 |
| Aclonifen[(c)] | 2383 | 89 | 418 | 18 | 82 | 33 | 1 | 22 | 66 | 34 | 261 | 2677 | 701 | 1976 | 74 |
| Diflufenican[(c)] | 1551 | 72 | 493 | 32 | 68 | 192 | 9 | 141 | 73 | 27 | 407 | 2150 | 1041 | 1109 | 52 |
| Tebuconazole[(c)] | 1084 | 54 | 323 | 30 | 70 | 170 | 9 | 97 | 57 | 43 | 748 | 2002 | 1167 | 835 | 42 |
| Mefenpyr-dietyl[(c)] | 316 | 71 | 99 | 31 | 69 | 36 | 8 | 30 | 83 | 17 | 91 | 443 | 220 | 223 | 50 |
| Epoxiconazole[(c)] | 13699 | 19 | 2757 | 20 | 80 | 1643 | 2 | 386 | 23 | 77 | 56629 | 71972 | 59771 | 12200 | 17 |
| Mean (pesticides) | | 46 | | 26 | 74 | | 10 | | 46 | 54 | | | | | 42 |
| Median (pesticides) | | 45 | | 26 | 74 | | 8 | | 54 | 46 | | | | | 42 |

**Table A 25: 2008 – 2009: Artificial wetland (AW) and forest buffer (FB) mass balances for the sixteen pesticides belonging to the analytical method. WSout, in and out stand for "watershed outlet", "inlet" and "outlet", respectively. [(a)] Water volumes are given in $m^3$. [(b)]Suspended sediments and nitrate units are kg. [(c)]Pesticide loads are in mg. [(d)]Reduction corresponded to the portion of pesticides that was actually dissipated through the two buffer zones and did not reach the stream.**

| Molecule | 2009 - 2010 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AWin | | AWout | | Annual reduction in AW | FBin | | FBout | | Annual reduction in FB | Ditch | WSout | Stream | Reduction[(d)] | |
| | load | AWin/ WSout (%) | load | AWout/ AWin (%) | % | load | FBin/ WSout (%) | load | FBout/ FBin (%) | % | load | load | load | load | %WSout |
| Water volumes[(a)] | 53269 | 86 | 38312 | 72 | 28 | 962 | 2 | | | | 7972 | 62203 | 46284 | 15919 | 26 |
| Suspended sediments[(b)] | 8274 | 80 | 6108 | 74 | 26 | 246 | 2 | | | | 1865 | 10385 | 7973 | 2412 | 23 |
| Nitrate[(b)] | 2007 | 86 | 1461 | 73 | 27 | 32 | 1 | | | | 288 | 2327 | 1749 | 579 | 25 |
| Isoproturon[(c)] | 39105 | 82 | 7437 | 19 | 81 | 1545 | 3 | | | | 7117 | 47767 | 14554 | 33213 | 70 |
| Chlorotoluron[(c)] | 342660 | 89 | 45649 | 13 | 87 | 6169 | 2 | | | | 37418 | 386246 | 83067 | 303180 | 78 |
| Atrazine[(c)] | 271 | 82 | 231 | 85 | 15 | 7 | 2 | | | | 51 | 329 | 282 | 47 | 14 |
| Chlorothalonil[(c)] | 1692 | 77 | 0 | 0 | 100 | 59 | 3 | | | | 441 | 2192 | 441 | 1751 | 80 |
| Prosulfocarb[(c)] | 2217 | 88 | 299 | 13 | 87 | 43 | 2 | | | | 269 | 2529 | 568 | 1961 | 78 |
| Fenpropidine[(c)] | 540 | 86 | 356 | 66 | 34 | 10 | 2 | | | | 80 | 629 | 436 | 193 | 31 |
| Ethofumesate[(c)] | 17 | 100 | 38 | 227 | *-127* | 0 | 0 | | | | 0 | 17 | 38 | -21 | -126 |
| S-metolachlor[(c)] | 0 | | 0 | | | 0 | | | | | 0 | 0 | 0 | 0 | |
| Metazachlor[(c)] | 4880 | 90 | 1192 | 24 | 76 | 46 | 1 | | | | 484 | 5411 | 1676 | 3735 | 69 |
| Napropamide[(c)] | 1912 | 80 | 765 | 40 | 60 | 56 | 2 | | | | 421 | 2389 | 1186 | 1203 | 50 |
| Cyproconazole[(c)] | 4925 | 85 | 2313 | 47 | 53 | 95 | 2 | | | | 797 | 5818 | 3111 | 2707 | 47 |
| Aclonifen[(c)] | 0 | | 0 | | | 0 | | | | | 0 | 0 | 0 | 0 | |
| Diflufenican[(c)] | 931 | 84 | 356 | 38 | 62 | 19 | 2 | | | | 159 | 1110 | 515 | 594 | 54 |
| Tebuconazole[(c)] | 86 | 100 | 114 | 133 | *-33* | 0 | 0 | | | | 0 | 86 | 114 | -28 | -33 |
| Mefenpyr-dietyl[(c)] | 452 | 84 | 587 | 130 | *-30* | 9 | 2 | | | | 80 | 541 | 667 | -126 | -23 |
| Epoxiconazole[(c)] | 1061 | 86 | 712 | 67 | 33 | 19 | 2 | | | | 159 | 1239 | 871 | 368 | 30 |
| Mean (pesticides) | | 86 | | 65 | 35 | | 2 | | | | | | | | 30 |
| Median (pesticides) | | 85 | | 43 | 57 | | 2 | | | | | | | | 48 |

**Table A 26: 2009 – 2010: Artificial wetland (AW) and forest buffer (FB) mass balances for the sixteen pesticides belonging to the analytical method. WSout, in and out stand for "watershed outlet", "inlet" and "outlet", respectively. [(a)] Water volumes are given in $m^3$. [(b)]Suspended sediments and nitrate units are kg. [(c)]Pesticide loads are in mg. [(d)]Reduction corresponded to the portion of pesticides that was actually dissipated through the two buffer zones and did not reach the stream.**

| Molecule | 2007 - 2008 Artificial Wetlands | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Drainage initiation | | | | | Intense drainage season | | | | | End of drainage | | | | |
| | 1 Oct. 2007 - 07 Dec. 2007 | | | | | 07 Dec. 2007 - 28 Apr. 2008 | | | | | 28 Apr. 2008 - 30 Sept. 2008 | | | | |
| | WSout load | AWin load | AWin/ Wsout (%) | AWout load | $\eta_{Lj}$ (%) | WSout load | AWin load | AWin/ Wsout (%) | AWout load | $\eta_{Lj}$ (%) | WSout load | AWin load | AWin/ Wsout (%) | AWout load | $\eta_{Lj}$ (%) |
| Water volume ($m^3$) | 10840 | 4842 | 45 | 1354 | 72 | 74958 | 23061 | 31 | 9809 | 57 | 23394 | 16321 | 70 | 7252 | 56 |
| Suspended sediments (g) | 904806 | 151704 | 17 | 60450 | 60 | 13285581 | 4280912 | 32 | 776077 | 82 | 5364744 | 2925585 | 55 | 1009677 | 65 |
| Nitrate (g) | 467989 | 78465 | 17 | 78897 | -1 | 3777993 | 1047931 | 28 | 369512 | 65 | 554795 | 426456 | 77 | 144338 | 66 |
| Isoproturon (mg) | 47452 | 18593 | 39 | 9631 | 48 | 131910 | 50444 | 38 | 69338 | -37 | 9346 | 6677 | 71 | 1140 | 83 |
| Chlorotoluron (mg) | 47672 | 23116 | 48 | 3728 | 84 | 96695 | 31037 | 32 | 14045 | 55 | 31017 | 23215 | 75 | 3488 | 85 |
| Atrazine (mg) | 1810 | 303 | 17 | 0 | 100 | 14 | 14 | | 0 | | 61 | 46 | 75 | 8 | 83 |
| Chlorothalonil (mg) | 0 | 0 | | 0 | | 1443 | 865 | | 172 | | 1719 | 1163 | 68 | 597 | 49 |
| Prosulfocarb (mg) | 5170 | 867 | 17 | 0 | 100 | 3874 | 1333 | 34 | 0 | 100 | 61 | 46 | 75 | 5 | 88 |
| Fenpropidine (mg) | 108 | 48 | 45 | 0 | 100 | 73 | 32 | 44 | 2 | 95 | 102 | 82 | 81 | 60 | 27 |
| Ethofumesate (mg) | 6607 | 1108 | 17 | 0 | 100 | 402 | 212 | 53 | 34 | 84 | 3700 | 3138 | 85 | 70 | 98 |
| S-metolachlor (mg) | 72 | 12 | 17 | 0 | 100 | 158 | 100 | | 17 | | 226 | 155 | 69 | 62 | 60 |
| Metazachlor (mg) | 25877 | 11945 | 46 | 2104 | 82 | 151779 | 46578 | 31 | 18224 | 61 | 60893 | 38906 | 64 | 8993 | 77 |
| Napropamide (mg) | 108 | 48 | 45 | 3 | 94 | 664 | 220 | 33 | 61 | 72 | 193 | 126 | 66 | 16 | 88 |
| Cyproconazole (mg) | 0 | 0 | | 0 | | 407 | 191 | | 133 | | 76 | 75 | 98 | 521 | -597 |
| Aclonifen (mg) | 144 | 24 | 17 | 0 | 100 | 0 | 0 | | 0 | | 0 | 0 | | 55 | |
| Diflufenican (mg) | 108 | 48 | 45 | 13 | 73 | 2086 | 1035 | 50 | 216 | 79 | 1622 | 1235 | 76 | 110 | 91 |
| Tebuconazole (mg) | 7764 | 1362 | 18 | 0 | 100 | 4311 | 1586 | 37 | 61 | 96 | 2424 | 1927 | 79 | 347 | 82 |
| Mefenpyr-dietyl (mg) | 15698 | 2632 | 17 | 0 | 100 | 299 | 94 | 31 | 26 | 72 | 95 | 79 | 83 | 12 | 85 |
| Epoxiconazole (mg) | 217 | 97 | 45 | 17 | 82 | 6181 | 4377 | 71 | 247 | 94 | 7047 | 4842 | 69 | 3162 | 35 |
| Mean (pesticides) | | | 31 | | 90 | | | 41 | | 70 | | | 76 | | 29 |
| Median (pesticides) | | | 28 | | 100 | | | 37 | | 79 | | | 75 | | 83 |

**Table A 27: Mass balances for artificial wetlands during 2007 – 2008 three identified drainage periods.**

| Molecule | 2007 - 2008 Forest Buffer | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Drainage initiation | | | | | Intense drainage season | | | | | End of drainage | | | | |
| | 1 Oct. 2007 - 07 Dec. 2007 | | | | | 07 Dec. 2007 - 28 Apr. 2008 | | | | | 28 Apr. 2008 - 30 Sept. 2008 | | | | |
| | WSout load | FBin load | FBin/ WSout (%) | FBout load | $\eta_{Lj}$ (%) | WSout load | FBin load | FBin/ WSout (%) | FBout load | $\eta_{Lj}$ (%) | WSout load | FBin load | FBin/ WSout (%) | FBout load | $\eta_{Lj}$ (%) |
| Water volume ($m^3$) | 10840 | 28 | 0 | 0 | 100 | 74958 | 11315 | 15 | 7765 | 31 | 23394 | 308 | 1 | 991 | -222 |
| Suspended sediments (g) | 904806 | 932 | 0 | 0 | 100 | 13285581 | 1461642 | 11 | 531924 | 64 | 5364744 | 64013 | 1 | 64280 | 0 |
| Nitrate (g) | 467989 | 482 | 0 | 0 | 100 | 3777993 | 743385 | 20 | 541833 | 27 | 554795 | 5995 | 1 | 5101 | 15 |
| Isoproturon (mg) | 47452 | 109 | 0 | 0 | 100 | 131910 | 42371 | 32 | 33747 | 20 | 9346 | 131 | 1 | 133 | -1 |
| Chlorotoluron (mg) | 47672 | 135 | 0 | 0 | 100 | 96695 | 19623 | 20 | 8106 | 59 | 31017 | 404 | 1 | 27 | 93 |
| Atrazine (mg) | 1810 | 2 | 0 | 0 | 100 | 14 | 0 | | 0 | | 61 | 0 | 0 | 0 | |
| Chlorothalonil (mg) | 0 | 0 | | 0 | | 1443 | 17 | | 0 | | 1719 | 28 | 2 | 0 | |
| Prosulfocarb (mg) | 5170 | 5 | 0 | 0 | 100 | 3874 | 2017 | 52 | 0 | 100 | 61 | 0 | 0 | 0 | 100 |
| Fenpropidine (mg) | 108 | 0 | 0 | 0 | 100 | 73 | 32 | 44 | 25 | 21 | 102 | 0 | 0 | 0 | 100 |
| Ethofumesate (mg) | 6607 | 7 | 0 | 0 | 100 | 402 | 63 | 16 | 0 | 100 | 3700 | 62 | 2 | 0 | 100 |
| S-metolachlor (mg) | 72 | 0 | 0 | 0 | 100 | 158 | 2 | | 0 | | 226 | 3 | 1 | 0 | |
| Metazachlor (mg) | 25877 | 70 | 0 | 0 | 100 | 151779 | 23683 | 16 | 17795 | 25 | 60893 | 631 | 1 | 182 | 71 |
| Napropamide (mg) | 108 | 0 | 0 | 0 | 100 | 664 | 112 | 17 | 78 | 31 | 193 | 3 | 1 | 1 | 58 |
| Cyproconazole (mg) | 0 | 0 | | 0 | | 407 | 71 | | 253 | | 76 | 2 | 2 | 0 | |
| Aclonifen (mg) | 144 | 0 | 0 | 0 | 100 | 0 | 0 | | 0 | | 0 | 0 | | 0 | |
| Diflufenican (mg) | 108 | 0 | 0 | 0 | 100 | 2086 | 265 | 13 | 78 | 71 | 1622 | 21 | 1 | 2 | 93 |
| Tebuconazole (mg) | 7764 | 8 | 0 | 0 | 100 | 4311 | 2055 | 48 | 578 | 72 | 2424 | 15 | 1 | 0 | 100 |
| Mefenpyr-dietyl (mg) | 15698 | 16 | 0 | 0 | 100 | 299 | 38 | 13 | 0 | 100 | 95 | 1 | 1 | 0 | 100 |
| Epoxiconazole (mg) | 217 | 1 | 0 | 0 | 100 | 6181 | 115 | 2 | 51 | 56 | 7047 | 55 | 1 | 2 | 96 |
| Mean (pesticides) | | | 0 | | 100 | | | 25 | | 59 | | | 1 | | 83 |
| Median (pesticides) | | | 0 | | 100 | | | 17 | | 59 | | | 1 | | 96 |

**Table A 28: Mass balances for forest buffer during 2007 – 2008 three identified drainage periods.**

| Molecule | 2008 - 2009 Artificial Wetlands | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Drainage initiation | | | | | Intense drainage season | | | | | End of drainage | | | | |
| | 1 Oct. 2008 - 02 Dec. 2008 | | | | | 02 Dec. 2008 - 26 Mar. 2009 | | | | | 26 Mar. 2009 - 30 Sept. 2009 | | | | |
| | WSout load | AWin load | AWin/ WSout (%) | AWout load | $\eta_{Lj}$ (%) | WSout load | AWin load | AWin/ WSout (%) | AWout load | $\eta_{Lj}$ (%) | WSout load | AWin load | AWin/ WSout (%) | AWout load | $\eta_{Lj}$ (%) |
| Water volume ($m^3$) | 6252 | 2591 | 41 | 0 | 100 | 50696 | 10835 | 21 | 5627 | 48 | 6104 | 5830 | 96 | 2731 | 53 |
| Suspended sediments (g) | 20340335 | 7683117 | 38 | 0 | 100 | 14017228 | 1119764 | 8 | 131359 | 88 | 693565 | 664202 | 96 | 107302 | 84 |
| Nitrate (g) | 305945 | 126474 | 41 | 0 | 100 | 1867908 | 628334 | 34 | 60421 | 90 | 213683 | 205252 | 96 | 122115 | 41 |
| Isoproturon (mg) | 63 | 26 | 41 | 0 | 100 | 977834 | 92048 | 9 | 51976 | 44 | 3579 | 3387 | 95 | 4218 | -25 |
| Chlorotoluron (mg) | 378 | 157 | 42 | 0 | 100 | 46139 | 10545 | 23 | 3494 | 67 | 424 | 397 | 94 | 284 | 29 |
| Atrazine (mg) | 0 | 0 | | 0 | | 176 | 74 | 42 | 13 | 83 | 30 | 29 | 96 | 16 | 43 |
| Chlorothalonil (mg) | 625 | 259 | 41 | 0 | 100 | 3464 | 428 | 12 | 112 | 74 | 322 | 301 | 94 | 126 | 58 |
| Prosulfocarb (mg) | 0 | 0 | | 0 | | 0 | 0 | | 0 | | 111 | 105 | 95 | 12 | 88 |
| Fenpropidine (mg) | 0 | 0 | | 0 | | 405 | 68 | 17 | 17 | 74 | 162 | 156 | 96 | 31 | 80 |
| Ethofumesate (mg) | 125 | 52 | 41 | 0 | 100 | 341 | 33 | 10 | 15 | 54 | 252 | 239 | 95 | 24 | 90 |
| S-metolachlor (mg) | 474 | 275 | 58 | 0 | 100 | 149 | 58 | 39 | 4 | 93 | 132 | 125 | 95 | 12 | 90 |
| Metazachlor (mg) | 4113 | 1709 | 42 | 0 | 100 | 26805 | 4895 | 18 | 1354 | 72 | 2034 | 1954 | 96 | 1352 | 31 |
| Napropamide (mg) | 256 | 107 | 42 | 0 | 100 | 163 | 68 | 42 | 13 | 81 | 27 | 26 | 98 | 16 | 37 |
| Cyproconazole (mg) | 0 | 0 | | 0 | | 2435 | 111 | 5 | 0 | 100 | 489 | 469 | 96 | 269 | 43 |
| Aclonifen (mg) | 37 | 16 | 42 | 0 | 100 | 189 | 32 | 17 | 15 | 52 | 2451 | 2335 | 95 | 403 | 83 |
| Diflufenican (mg) | 229 | 95 | 42 | 0 | 100 | 572 | 173 | 30 | 55 | 68 | 1349 | 1283 | 95 | 438 | 66 |
| Tebuconazole (mg) | 125 | 52 | 41 | 0 | 100 | 1005 | 208 | 21 | 41 | 80 | 872 | 824 | 95 | 282 | 66 |
| Mefenpyr-dietyl (mg) | 19 | 8 | 42 | 0 | 100 | 152 | 49 | 33 | 12 | 76 | 273 | 259 | 95 | 87 | 67 |
| Epoxiconazole (mg) | 125 | 52 | 41 | 0 | 100 | 63756 | 5859 | 9 | 795 | 86 | 8090 | 7789 | 96 | 2943 | 62 |
| Mean (pesticides) | | | 43 | | 100 | | | 22 | | 74 | | | 95 | | 57 |
| Median (pesticides) | | | 42 | | 100 | | | 18 | | 74 | | | 95 | | 64 |

**Table A 29: Mass balances for artificial wetlands during 2008 – 2009 three identified drainage periods.**

| Molecule | 2008 - 2009 Forest Buffer | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Drainage initiation | | | | | Intense drainage season | | | | | End of drainage | | | | |
| | 1 Oct. 2008 - 02 Dec. 2008 | | | | | 02 Dec. 2008 - 26 Mar. 2009 | | | | | 26 Mar. 2009 - 30 Sept. 2009 | | | | |
| | WSout load | FBin load | FBin/ WSout (%) | FBout load | $\eta_{Lj}$ (%) | WSout load | FBin load | FBin/ WSout (%) | FBout load | $\eta_{Lj}$ (%) | WSout load | FBin load | FBin/ WSout (%) | FBout load | $\eta_{Lj}$ (%) |
| Water volume ($m^3$) | 6252 | 3662 | 59 | 2259 | 38 | 50696 | 4326 | 9 | 3451 | 20 | 6104 | 141 | 2 | 145 | -3 |
| Suspended sediments (g) | 20340335 | 460421 | 2 | 408534 | 11 | 14017228 | 632677 | 5 | 304853 | 52 | 693565 | 13148 | 2 | 16256 | -24 |
| Nitrate (g) | 305945 | 179524 | 59 | 103016 | 43 | 1867908 | 191771 | 10 | 99253 | 48 | 213683 | 1741 | 1 | 477 | 73 |
| Isoproturon (mg) | 63 | 37 | 59 | 45 | -22 | 977834 | 15679 | 2 | 233 | 99 | 3579 | 88 | 2 | 76 | 14 |
| Chlorotoluron (mg) | 378 | 221 | 58 | 45 | 80 | 46139 | 2621 | 6 | 31 | 99 | 424 | 14 | 3 | 3 | 79 |
| Atrazine (mg) | 0 | 0 | | 0 | | 176 | 37 | 21 | 22 | 40 | 30 | 1 | 2 | 0 | 100 |
| Chlorothalonil (mg) | 625 | 366 | 59 | 226 | 38 | 3464 | 78 | 2 | 24 | 69 | 322 | 11 | 4 | 15 | -27 |
| Prosulfocarb (mg) | 0 | 0 | | 0 | | 0 | 0 | | 0 | | 111 | 1 | 1 | 0 | 100 |
| Fenpropidine (mg) | 0 | 0 | | 5 | | 405 | 39 | 10 | 40 | 0 | 162 | 1 | 1 | 0 | 100 |
| Ethofumesate (mg) | 125 | 73 | 59 | 36 | 51 | 341 | 7 | 2 | 5 | 30 | 252 | 1 | 1 | 2 | -15 |
| S-metolachlor (mg) | 474 | 37 | 8 | 18 | 51 | 149 | 35 | 24 | 0 | 100 | 132 | 1 | 1 | 0 | 100 |
| Metazachlor (mg) | 4113 | 2404 | 58 | 807 | 66 | 26805 | 1781 | 7 | 470 | 74 | 2034 | 29 | 1 | 0 | 100 |
| Napropamide (mg) | 256 | 149 | 58 | 18 | 88 | 163 | 35 | 22 | 22 | 38 | 27 | 0 | 0 | 0 | 100 |
| Cyproconazole (mg) | 0 | 0 | | 35 | | 2435 | 44 | 2 | 0 | 100 | 489 | 7 | 1 | 0 | 100 |
| Aclonifen (mg) | 37 | 22 | 58 | 9 | 57 | 189 | 4 | 2 | 5 | -35 | 2451 | 8 | 0 | 8 | 3 |
| Diflufenican (mg) | 229 | 134 | 58 | 55 | 59 | 572 | 44 | 8 | 72 | -64 | 1349 | 14 | 1 | 14 | 5 |
| Tebuconazole (mg) | 125 | 73 | 59 | 45 | 38 | 1005 | 86 | 9 | 49 | 43 | 872 | 11 | 1 | 3 | 73 |
| Mefenpyr-dietyl (mg) | 19 | 11 | 58 | 5 | 57 | 152 | 24 | 16 | 24 | -2 | 273 | 1 | 0 | 1 | 32 |
| Epoxiconazole (mg) | 125 | 73 | 59 | 45 | 38 | 63756 | 1467 | 2 | 549 | 63 | 8090 | 103 | 1 | 296 | -188 |
| Mean (pesticides) | | | 54 | | 50 | | | 9 | | 43 | | | 1 | | 42 |
| Median (pesticides) | | | 58 | | 54 | | | 7 | | 43 | | | 1 | | 76 |

**Table A 30: Mass balances for forest buffer during 2008 – 2009 three identified drainage periods.**

| Molecule | 2009 - 2010 Artificial Wetlands | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Drainage initiation | | | | | Intense drainage season | | | | | End of drainage | | | | |
| | 1 Oct. 2009 - 28 Dec. 2009 | | | | | 28 Dec. 2009 - 18 Mar. 2010 | | | | | 18 Mar. 2010 - 11 May 2010 | | | | |
| | WSout load | AWin load | AWin/ WSout (%) | AWout load | $\eta_{Lj}$ (%) | WSout load | AWin load | AWin/ WSout (%) | AWout load | $\eta_{Lj}$ (%) | WSout load | AWin load | AWin/ WSout (%) | AWout load | $\eta_{Lj}$ (%) |
| Water volume ($m^3$) | 2080 | 2017 | 97 | 0 | 100 | 54115 | 45253 | 84 | 36412 | 20 | 6008 | 5999 | 100 | 1900 | 68 |
| Suspended sediments (g) | 143057 | 143057 | 100 | 0 | 100 | 9688169 | 7578270 | 78 | 6008469 | 21 | 553932 | 553082 | 100 | 99488 | 82 |
| Nitrate (g) | 41457 | 41457 | 100 | 0 | 100 | 2109019 | 1788843 | 85 | 1399832 | 22 | 176964 | 176739 | 100 | 60886 | 66 |
| Isoproturon (mg) | 28976 | 23684 | 82 | 0 | 100 | 16339 | 12975 | 79 | 6546 | 50 | 2452 | 2446 | 100 | 891 | 64 |
| Chlorotoluron (mg) | 225980 | 213380 | 94 | 0 | 100 | 154965 | 123989 | 80 | 43645 | 65 | 5301 | 5291 | 100 | 2004 | 62 |
| Atrazine (mg) | 12 | 12 | 100 | 0 | 100 | 303 | 245 | 81 | 231 | 6 | 15 | 15 | 100 | 0 | 100 |
| Chlorothalonil (mg) | 0 | 0 | | 0 | | 2022 | 1522 | 75 | 0 | 100 | 171 | 170 | 100 | 0 | 100 |
| Prosulfocarb (mg) | 1351 | 1266 | 94 | 0 | 100 | 1135 | 908 | 80 | 299 | 67 | 43 | 43 | 100 | 0 | 100 |
| Fenpropidine (mg) | 43 | 42 | 99 | 0 | 100 | 526 | 438 | 83 | 337 | 23 | 60 | 60 | 100 | 19 | 68 |
| Ethofumesate (mg) | 0 | 0 | | 0 | | 0 | 0 | | 0 | | 17 | 17 | 100 | 38 | -127 |
| S-metolachlor (mg) | 0 | 0 | | 0 | | 0 | 0 | | 0 | | 0 | 0 | | 66096 | |
| Metazachlor (mg) | 137 | 128 | 94 | 0 | 100 | 4195 | 3675 | 88 | 740 | 80 | 1079 | 1077 | 100 | 451 | 58 |
| Napropamide (mg) | 268 | 258 | 96 | 0 | 100 | 2019 | 1553 | 77 | 763 | 51 | 102 | 101 | 100 | 1129 | -1014 |
| Cyproconazole (mg) | 148 | 141 | 96 | 0 | 100 | 5262 | 4376 | 83 | 2309 | 47 | 408 | 408 | 100 | 3441 | -744 |
| Aclonifen (mg) | 0 | 0 | | 0 | | 0 | 0 | | 0 | | 0 | 0 | | 0 | |
| Diflufenican (mg) | 131 | 130 | 100 | 0 | 100 | 919 | 741 | 81 | 337 | 55 | 60 | 60 | 100 | 19 | 68 |
| Tebuconazole (mg) | 0 | 0 | | 0 | | 0 | 0 | | 76 | | 86 | 86 | 100 | 38 | 56 |
| Mefenpyr-dietyl (mg) | 15 | 14 | 96 | 0 | 100 | 526 | 438 | 83 | 231 | 47 | 0 | 0 | | 356 | |
| Epoxiconazole (mg) | 30 | 28 | 96 | 0 | 100 | 1052 | 875 | 83 | 674 | 23 | 157 | 157 | 100 | 38 | 76 |
| Mean (pesticides) | | | 95 | | 100 | | | 81 | | 51 | | | 100 | | -87 |
| Median (pesticides) | | | 96 | | 100 | | | 81 | | 50 | | | 100 | | 64 |

**Table A 31: Mass balances for artificial wetlands during 2009 – 2010 three identified drainage periods.**

| Molecule | 2009 - 2010 Forest Buffer | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Drainage initiation | | | | | Intense drainage season | | | | | End of drainage | | | | |
| | 1 Oct. 2009 - 28 Dec. 2009 | | | | | 28 Dec. 2009 - 18 Mar. 2010 | | | | | 18 Mar. 2010 - 11 May 2010 | | | | |
| | WSout load | FBin load | FBin/ WSout (%) | FBout load | $\eta_{Lj}$ (%) | WSout load | FBin load | FBin/ WSout (%) | FBout load | $\eta_{Lj}$ (%) | WSout load | FBin load | FBin/ WSout (%) | FBout load | $\eta_{Lj}$ (%) |
| Water volume ($m^3$) | 2080 | 14 | 1 | 0 | 100 | 54115 | 939 | 2 | 0 | 100 | 6008 | 9 | 0 | 0 | 100 |
| Suspended sediments (g) | 143057 | 0 | 0 | 0 | | 9688169 | 244884 | 3 | 0 | 100 | 553932 | 851 | 0 | 0 | 100 |
| Nitrate (g) | 41457 | 0 | 0 | 0 | | 2109019 | 32009 | 2 | 0 | 100 | 176964 | 225 | 0 | 0 | 100 |
| Isoproturon (mg) | 4021 | 1176 | 29 | 0 | 100 | 16339 | 364 | 2 | 0 | 100 | 2452 | 6 | 0 | 0 | 100 |
| Chlorotoluron (mg) | 164830 | 2800 | 2 | 0 | | 154965 | 3359 | 2 | 0 | 100 | 5301 | 10 | 0 | 0 | 100 |
| Atrazine (mg) | 11 | 0 | 0 | 0 | | 303 | 7 | 2 | 0 | 100 | 15 | 0 | 0 | 0 | |
| Chlorothalonil (mg) | 0 | 0 | | 0 | | 2022 | 59 | 3 | 0 | 100 | 171 | 0 | 0 | 0 | |
| Prosulfocarb (mg) | 913 | 19 | 2 | 0 | | 1135 | 24 | 2 | 0 | 100 | 43 | 0 | 0 | 0 | |
| Fenpropidine (mg) | 39 | 0 | 0 | 0 | | 526 | 9 | 2 | 0 | 100 | 268 | 0 | 0 | 0 | |
| Ethofumesate (mg) | 0 | 0 | | 0 | | 0 | 0 | | 0 | | 17 | 0 | 0 | 0 | |
| S-metolachlor (mg) | 1223 | 0 | 0 | 0 | | 9458 | 0 | 0 | 0 | | 1220 | 0 | 0 | 0 | |
| Metazachlor (mg) | 93 | 2 | 2 | 0 | | 4195 | 43 | 1 | 0 | 100 | 1079 | 2 | 0 | 0 | 100 |
| Napropamide (mg) | 214 | 2 | 1 | 0 | | 2019 | 53 | 3 | 0 | 100 | 102 | 0 | 0 | 0 | |
| Cyproconazole (mg) | 116 | 1 | 1 | 0 | | 5262 | 93 | 2 | 0 | 100 | 408 | 1 | 0 | 0 | 100 |
| Aclonifen (mg) | 0 | 0 | | 0 | | 0 | 0 | | 0 | | 0 | 0 | | 0 | |
| Diflufenican (mg) | 126 | 0 | 0 | 0 | | 919 | 19 | 2 | 0 | 100 | 60 | 0 | 0 | 0 | |
| Tebuconazole (mg) | 0 | 0 | | 0 | | 0 | 0 | | 0 | | 86 | 0 | 0 | 0 | |
| Mefenpyr-dietyl (mg) | 12 | 0 | 1 | 0 | | 526 | 9 | 2 | 0 | 100 | 0 | 0 | | 0 | |
| Epoxiconazole (mg) | 23 | 0 | 1 | 0 | | 1052 | 19 | 2 | 0 | 100 | 157 | 0 | 0 | 0 | |
| Mean (pesticides) | | | | | | | | 2 | 0 | 100 | | | 0 | | 100 |
| Median (pesticides) | | | | | | | | 2 | | 100 | | | 0 | | 100 |

**Table A 32: Mass balances for forest buffer during 2009 – 2010 three identified drainage periods.**

## Appendix IX Pesticide concentration at the inlet and outlet of the buffer zones

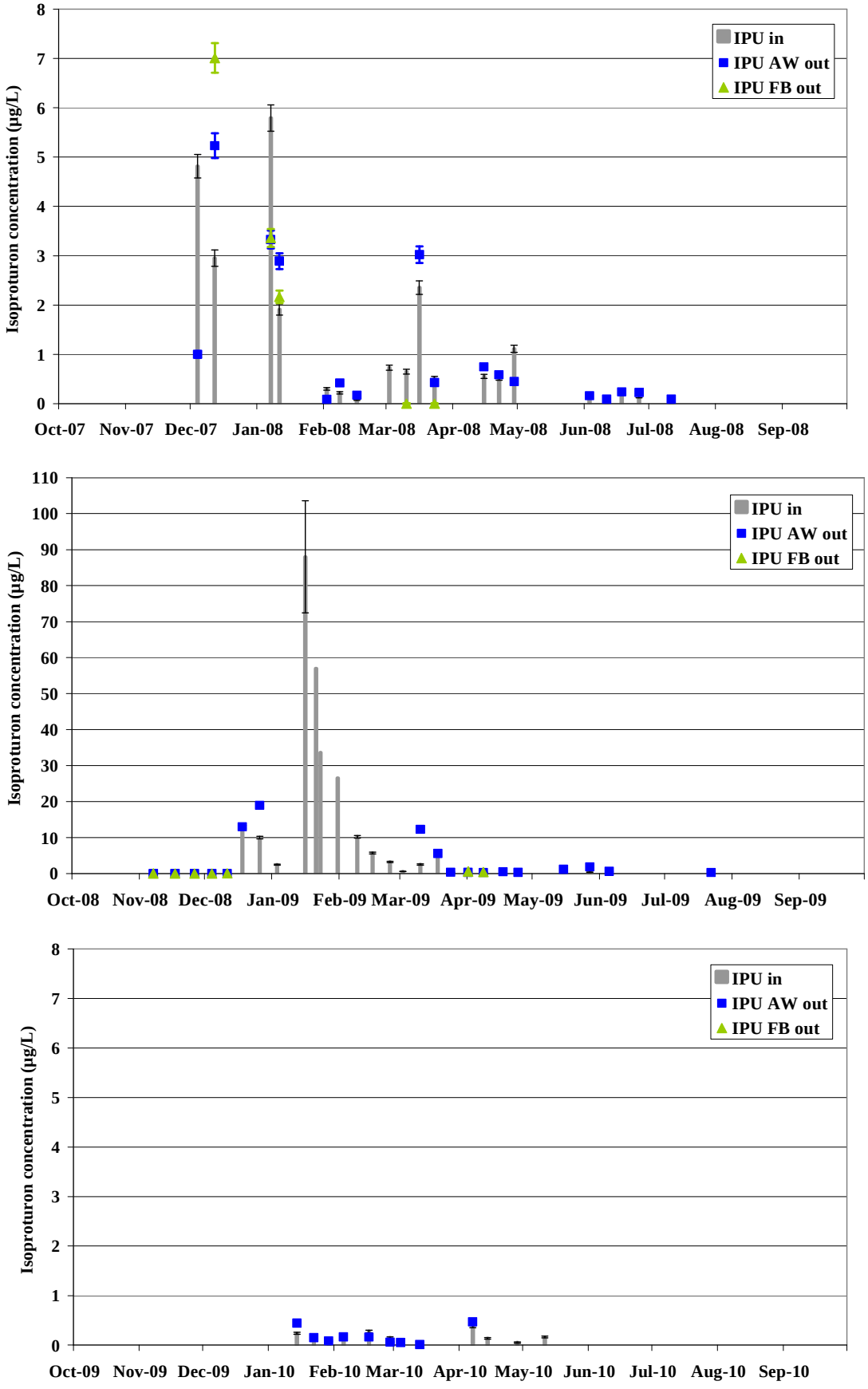


**Fig. A 3: Isoproturon (IPU) inlet (in) and artificial wetland (AW) and forest buffer (FB) outlet (out) concentrations.**

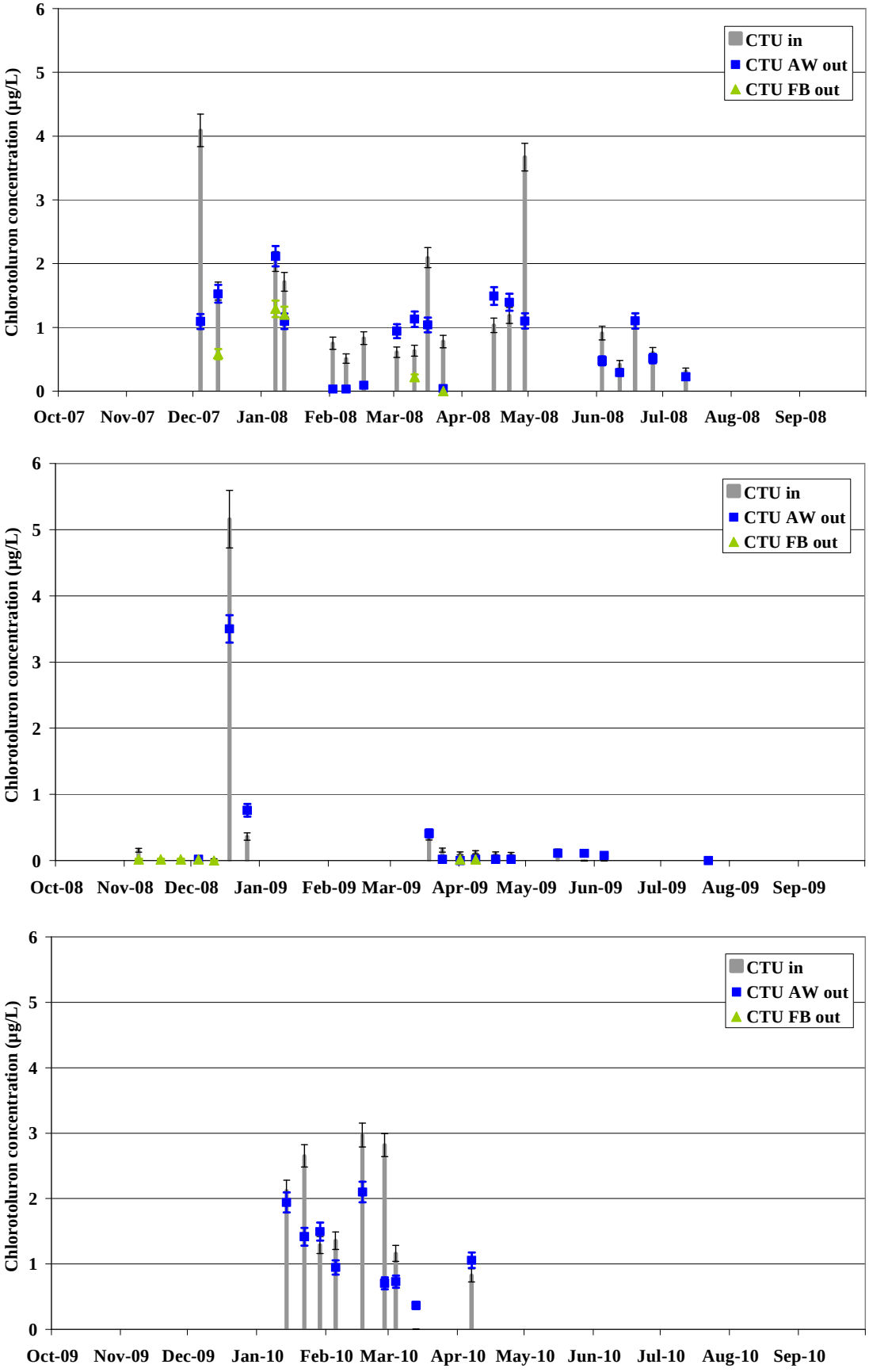


**Fig. A 4: Chlorotoluron (CTU) inlet (in) and artificial wetland (AW) and forest buffer (FB) outlet (out) concentrations.**

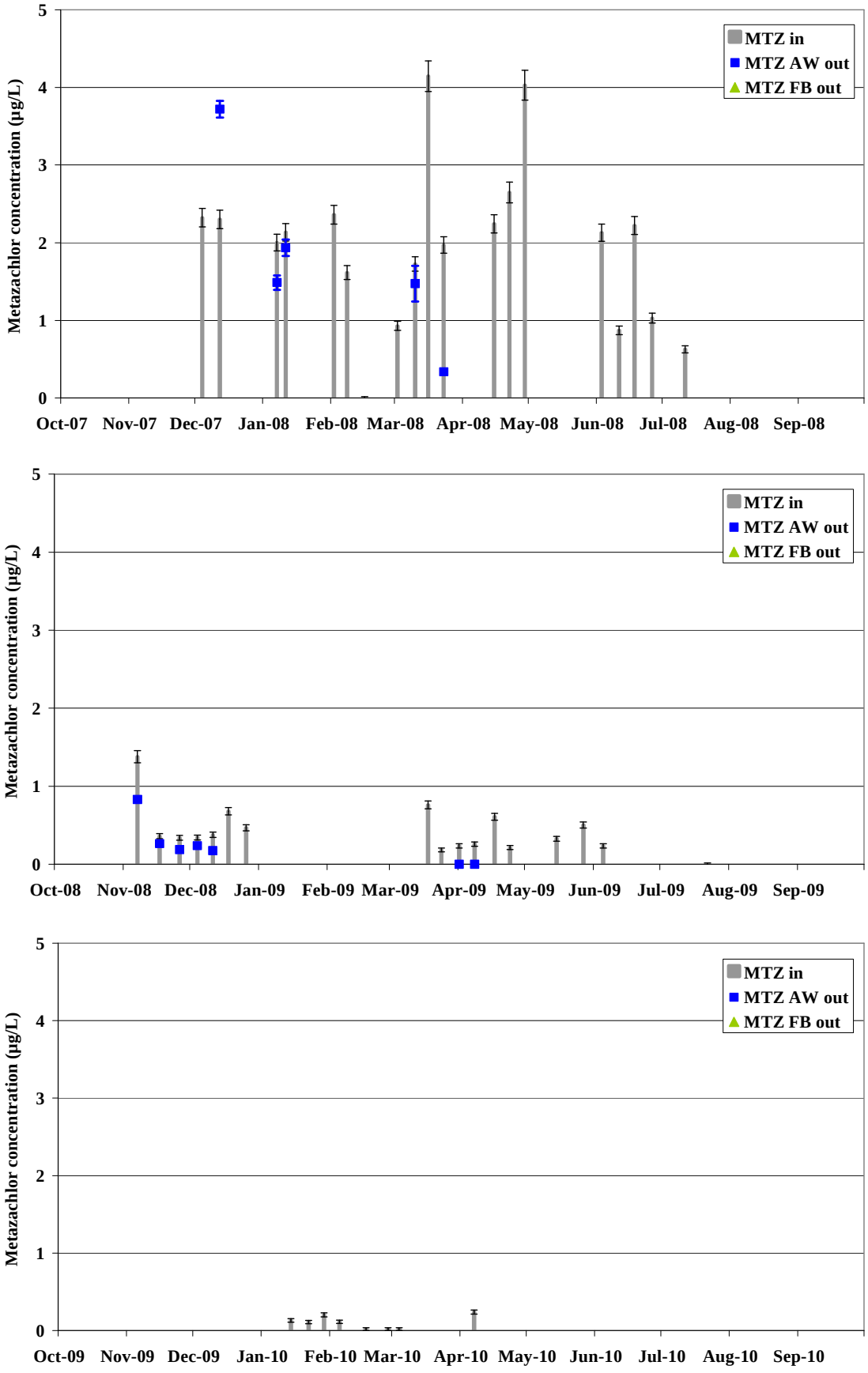


**Fig. A 5: Metazachlor (MTZ) inlet (in) and artificial wetland (AW) and forest buffer (FB) outlet (out) concentrations.**

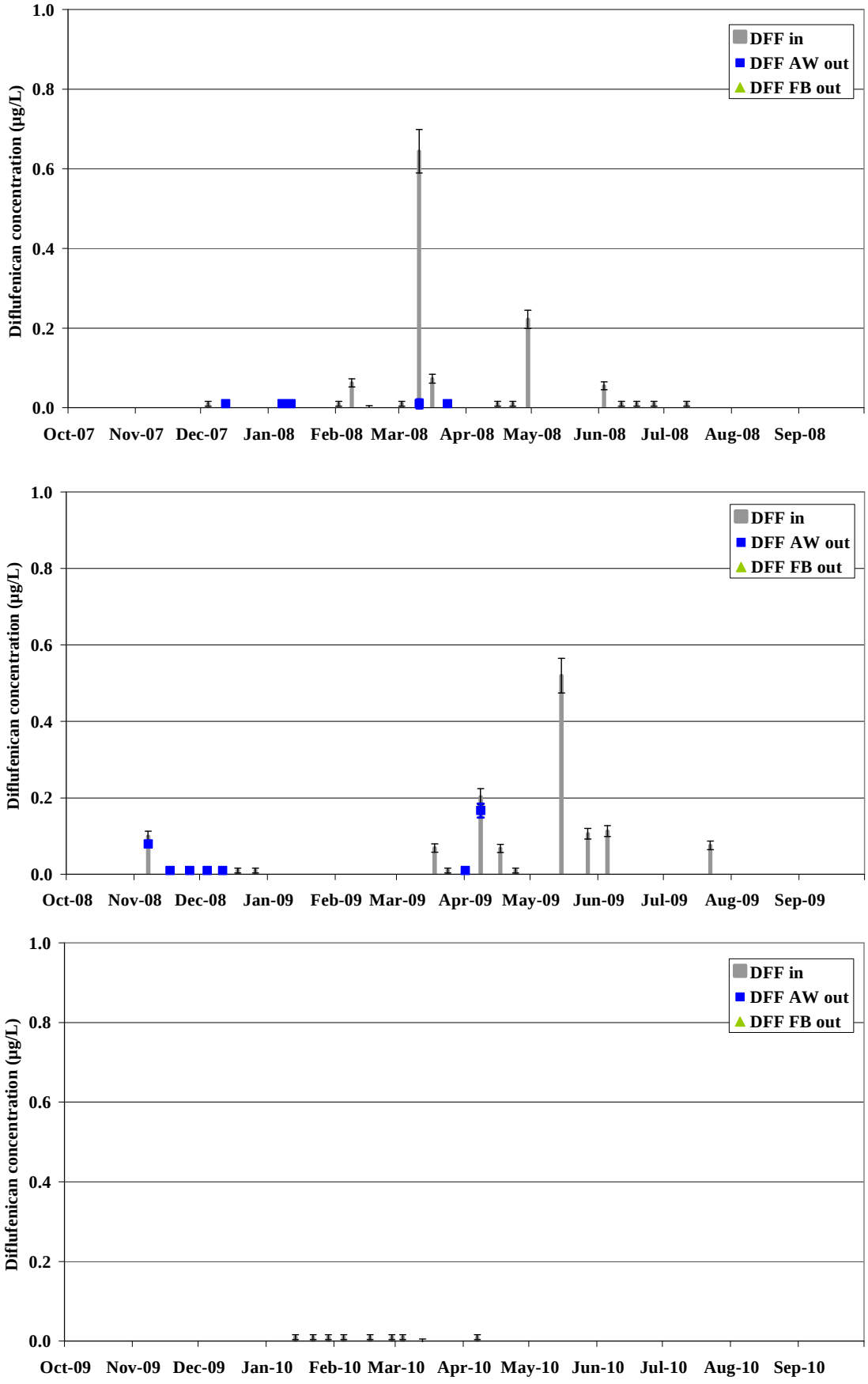


**Fig. A 6: Diflufenican (DFF) inlet (in) and artificial wetland (AW) and forest buffer (FB) outlet (out) concentrations.**

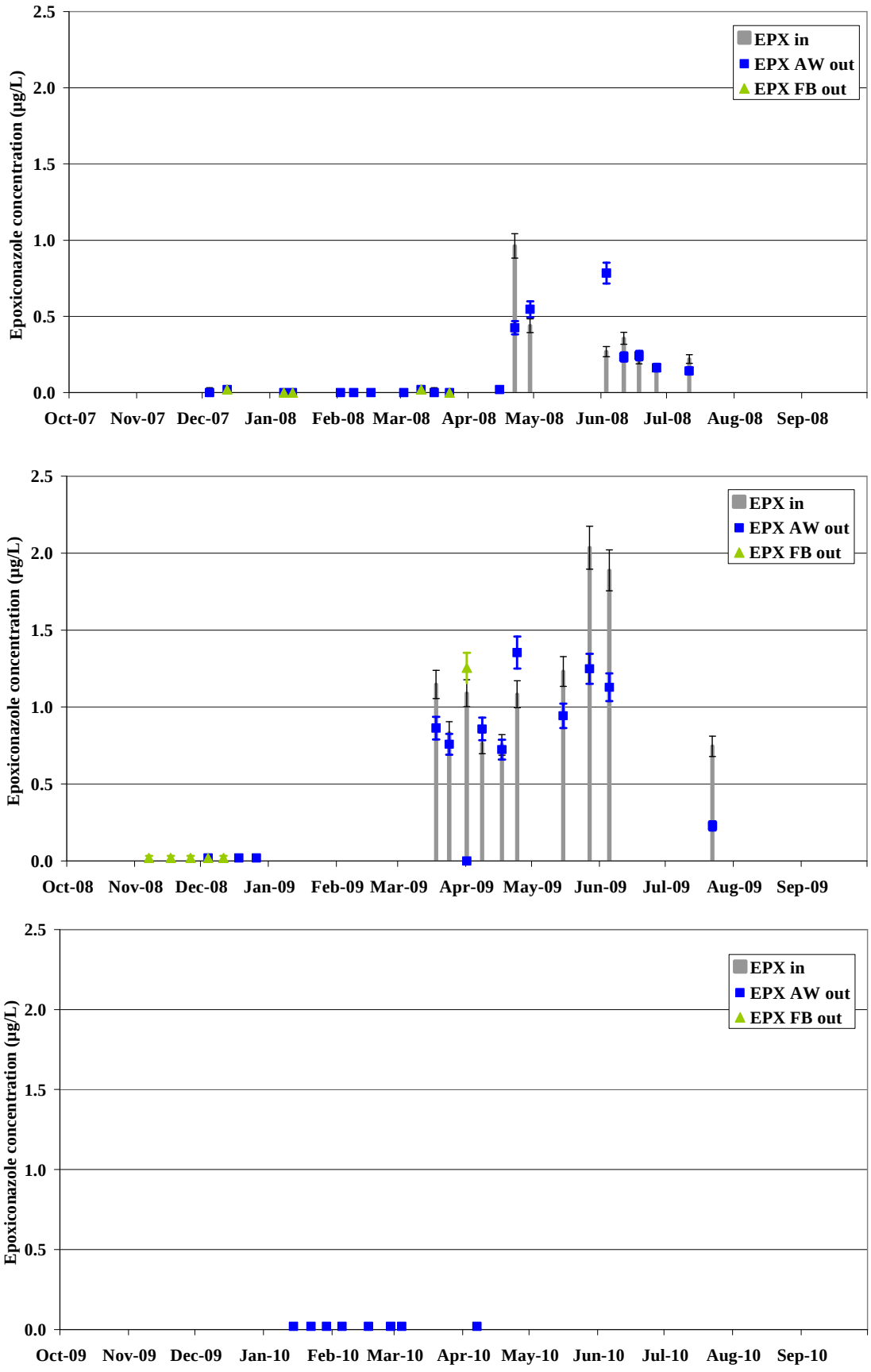


**Fig. A 7: Epoxiconazole (EPX) inlet (in) and artificial wetland (AW) and forest buffer (FB) outlet (out) concentrations.**

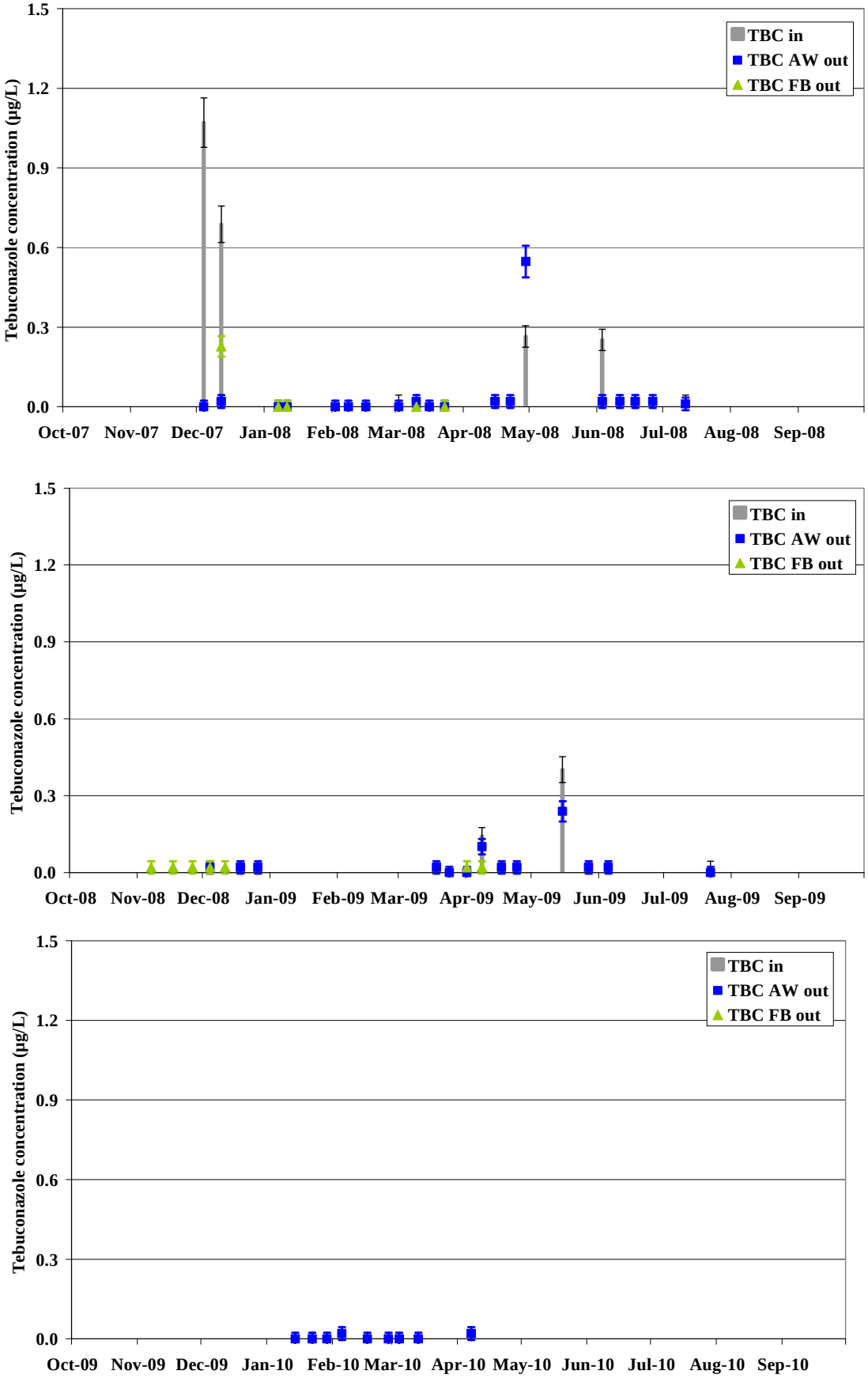


**Fig. A 8: Tebuconazole (TBC) inlet (in) and artificial wetland (AW) and forest buffer (FB) outlet (out) concentrations.**

## Appendix X Scientific communication concerning the results of this Ph.D project

## PUBLICATIONS

**In preparation :**

- **Passeport, E.**, Benoit, P., Bergheaud, V., Coquet, Y., Tournebize, J. Artificial wetland and forest buffer potential for adsorption and desorption of selected pesticides. *In Prep. possibly for Ecological Engineering.*
- **Passeport, E.**, Benoit, P., Bergheaud, V., Coquet, Y., Tournebize, J. Artificial wetland and forest buffer potential for epoxiconazole degradation. *In Prep. for Chemosphere.*
- Stehle, S., Elsaesser, D., Gregoire, C., **Passeport, E.**, Payraudau, S., Tournebize, J., Schulz, R. Pesticide risk mitigation by vegetated treatment: a Meta analysis. *In Prep.*
- Harris, L., Brush, M., Hanks, D., Hyman, J., Kaushal, S., D., Kellogg, Q., Mayer, P., **Passeport, E.**, Pole, J.C., Stander, E., Vidon, P., Youssef, M.. Plugging the nitrogen leaks: Enhancing watershed denitrification with ecological engineering. *In Prep for Ecological Engineering.*

**Published or In Press :**

- Lange J., Schütz T., Gregoire C., Elsässer D., Schulz R., **Passeport E**., Tournebize J. Multi-tracer experiments to characterize contaminant mitigation capacities for different types of artificial wetlands. *In Press. International Journal of Environmental Analytical Chemistry*.
- **Passeport, E.**, Guenne, A., Culhaoglu, T., Moreau, S., Bouyé, J.-M., Tournebize; J. (2010). Design of experiments and detailed uncertainty analysis to develop and validate a solid-phase microextraction/gas chromatography–mass spectrometry method for the simultaneous analysis of 16 pesticides in water. *Journal of Chromatography A.* 1217(33):5317-5327.
- **Passeport E**., Tournebize J., Jankowfsky S., Prömse B., Chaumont C., Coquet Y., Lange J. (2010). Artificial Wetland and Forest Buffer Zone: Hydraulic and Tracer Characterization. *Vadose Zone Journal*, 9:73-84, Published online 11 Jan. 2010. DOI:10.2136/vzj2008.0164.
- Gregoire C., Elsaesser D., Huguenot D., Lange J., Lebeau T., Merli A., Mose R., **Passeport E**., Payraudeau S., Schütz T., Schulz R., Tapia-Padilla G., Tournebize J., Trevisan M., Wanko A. (2009). Review: Mitigation of agricultural nonpoint-source pesticide pollution in artificial wetland ecosystems. *Environmental Chemistry Letters*, 7(3):205-231, DOI: 10.1007/s10311-008-0167-9.

## ORAL PRESENTATIONS

- **Passeport E**., Tournebize J., Lange J., Chaumont C. Non-point source pesticide pollution mitigation in buffer zones: a tracer experiment for hydrological characterization. 5$^{th}$ European Conference on Pesticides and Related Organic Micropollutants in the Environment, Marseille, France, 22-25 October 2008.
- **Passeport E**., Tournebize J., Jankowfsky S., Prömse B., Chaumont C., Coquet Y., Lange J. Artificial Wetland and Forest Buffer Zone: Hydraulic and Tracer Characterization. SWS-WWA-WBS joint meeting, Madison, WI, USA, 21-26 June 2009.
- **Passeport E.,** Tournebize J., Chaumont C., Bernard V., Margoum C., Gril J-J., Paineau F., Culhaoglu T., Guenne A., Coquet Y. Artificial wetland and forest buffer for pesticide pollution control. 3rd Wetland Pollutant Dynamics and Control Symposium WETPOL, Barcelona, Spain, 20-24 September, 2009.
- **Passeport E**., Tournebize, J., Chaumont, C., Bernard, V., Margoum, C., Gril, J.-J., Paineau, F., Culhaoglu, T., Guenne, A., Coquet, Y. Efficiency of an artificial wetland and a forest

buffer for pesticide mitigation in a subsurface drained watershed. SWS annual meeting, Salt Lake City, UT, USA, 28 June-2 July 2010.

POSTERS

- **Passeport E.,** Lange, J., Tournebize, J., Chaumont C., Jankowfsky, S., Proemse B., Schütz T., Blattmann E. Agricultural drained watershed effluents: a tracer experiment for hydraulic characterization of an artificial wetland as pesticide mitigation system. European Geosciences Union General Assembly, Vienna, Austria, 13-18 April 2008.
- **Passeport E.,** Chaumont C., Vincent B., Guenne A., Tournebize J. Agricultural non-point source nitrate and pesticide pollution mitigation through artificial wetlands. Denitrification RCN workshop, Denitrification in Managed Ecosystems, Coastal Institute, University of Rhode Island Narragansett, RI, USA, 12-14 May 2009.
- **Passeport E.,** Tournebize J., Chaumont C., Bernard V., Margoum C., Gril J-J., Paineau F., Culhaoglu T., Guenne A., Coquet Y. Efficiency of an artificial wetland and a forest buffer for pesticide mitigation. Ecological Engg from Concepts to Applications (EECA) International Congress, Paris, France, 2-4 December 2009.