

# Is it possible to extrapolate results of aquatic microcosm and mesocosm experiments with pesticides between climate zones in Europe?

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## 1 Introduction

Higher tier studies to assess the environmental risks of pesticides have been performed mainly in Atlantic Central Europe and North America and results of such studies have been extrapolated to other climatic regions such as South Europe (Ramos et al. 2000; López-Mancisidor et al. 2008a). Since climate (e.g., sun hours, rainfall, temperature), agroecosystems (e.g., crop rotation, field size), and edge-of-field surface waters (e.g., hydrology, ecology) in those regions are quite different, it may be expected that exposure profiles and effects of pesticides in surface waters are also different (Tarazona 2005; López-Mancisidor et al. 2008a). It may thus be questionable whether the extrapolation of results from higher tier studies over these regions is justified (Ramos et al. 2000; López-Mancisidor et al. 2008a). On the other hand, it is neither financially nor practically feasible to test a large number of chemicals on a large number of species and

communities in different localities (Brock et al. 2006). In the EU, these concerns were acknowledged with the recent adoption of a new regulation concerning the placing of plant protection products on the market (Regulation (EC) No. 1107/2009; EU 2009). According to this regulation, authorizations granted by one Member State should in principle be accepted by other Member States where agricultural, plant health, and environmental (including climatic) conditions are comparable. To this end, three zones with such comparable ecological and climatic conditions were distinguished, namely North, Central, and South (Table 1).

Van Wijngaarden et al. (2005a), Van den Brink et al. (2006), and Maltby et al. (2009) reviewed aquatic model ecosystem studies carried out with insecticides, herbicides, and fungicides, respectively. The vast majority of the European studies were conducted in the Central zone, whereas very few studies were reported for both the South and the North zone (Table 1). To account for the lack of studies in the South zone, a research project was initiated in 2010 that started with the set up of an outdoor microcosm facility in Lisbon (Portugal). One of the aims of this facility is to study the possibilities and limitations in the spatial extrapolation of regulatory acceptable concentrations (RACs) derived from outdoor micro-/mesocosm experiments between European climate zones. The present paper aims at summarizing differences in (1) ecology of edge-of-field surface waters, (2) exposure conditions to pesticides, (3) direct and indirect effects, and (4) recovery potential in pesticide stressed (semi-)field freshwater ecosystems under South zone compared to Central zone conditions that a priori may be anticipated. Implications for choices related to the experimental design of experiments in Portugal are discussed based on this analysis.

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**Table 1** Definition of zones for the authorisation of plant protection products as defined in annex I to (EC) No 1107/2009 (EU 2009)

	North	Central	South
Countries	Denmark Estonia Finland Latvia Lithuania Sweden	Austria Belgium Czech Republic Germany Hungary Ireland Luxembourg Netherlands Poland Romania Slovenia Slovakia United Kingdom	Bulgaria Cyprus France Greece Italy Malta Portugal Spain
No. of studies	2	38	2

For each zone, the total number of aquatic model ecosystem studies included in reviews by Van Wijngaarden et al. (2005a; insecticides), Van den Brink et al. (2006; herbicides) and Maltby et al. (2009; fungicides) is indicated

## 2 Ecology of edge-of-field surface waters

Edge-of-field surface waters in agricultural landscapes are subject to features such as climate, relief, geology, hydrology, and land use and related environmental stress. These features affect the species composition of communities and traits of the organisms present. According to Moss et al. (2004), Alvarez Cobelas et al. (2005), Bonada et al. (2007), and Prados and Novillo-Villajos (2010), some important ecological features of Mediterranean freshwaters are:

- Many Mediterranean aquatic environments are temporal, increasing the importance of adaptations by emergent and submersed vascular plants and macro-invertebrates to resilient environments (e.g., drought resistant life stages, well-developed dispersal capacity, short life-cycles)
- Macrophyte density may be greater in Mediterranean freshwaters as a result of higher temperatures and (hence) longer plant growing seasons, which may influence limnological functioning (e.g., nutrient recycling)
- The threshold of highest species richness of algae is shifted towards higher total phosphorous levels in Mediterranean freshwaters when compared to temperate freshwater ecosystems
- Local trait richness, diversity, and number of endemic species of macro-invertebrates may be higher in Mediterranean streams than in temperate streams
- Insect communities of permanent Mediterranean water courses are dominated by Ephemeroptera, Plecoptera, and Trichoptera and that of temporal Mediterranean

waters by Odonata, Coleoptera, and Heteroptera. Plecoptera and Trichoptera may have a higher local genus richness in temperate than in Mediterranean sites, whereas Odonata, Coleoptera, and Heteroptera may be richer in Mediterranean sites.

To address the ecological features described above when conducting and interpreting micro-/mesocosm experiments, it is recommended to develop ecological scenarios for typical Mediterranean freshwater ecosystems (e.g., Prados and Novillo-Villajos 2010). These scenarios should also address the diversity in species traits, i.e., physiological, morphological, and ecological attributes of species (Usseglio-Polatera et al. 2000). This will allow a comparison between the community/species composition of the model ecosystems in Lisbon and that of several natural freshwater ecosystems that these experimental ecosystems simulate. This information can then also be used to interpret and extrapolate treatment-related effects observed in (semi-)field experiments. The use of traits have indeed been proven in recent years to have great potential as community descriptors to determine quantitative links between pesticide toxicity and aquatic field community alterations (e.g., Baird and Van den Brink 2007; Liess et al. 2008; Van den Brink et al. 2010).

## 3 Exposure conditions to pesticides

The difference in climatic conditions between Mediterranean countries and central Europe will affect the fate of pesticides in many ways. The higher temperature and sunlight will increase the dissipation of pesticides, while the larger differences in elevation will lead to an increase in runoff (Daam and Van den Brink 2010). Runoff and soil erosion can be the largest contributors to pesticide surface water contamination in Mediterranean countries (Tarazona 2005), particularly after heavy rainfall following a period of drought. Soil particles and associated pesticides that enter freshwater ecosystems may result in longer-term exposure regimes, whereas spray drift will generally result in short-term pulsed exposure regimes. According to Brock et al. (2010), available monitoring data suggest that Forum for the Co-ordination of Pesticide Fate Models and Their Use (FOCUS 2001) simulations are able to reproduce the general characteristics of measured pesticide concentrations in the water column of edge-of-field water bodies. However, they recommend that further experimental and monitoring work is necessary to underpin the validity of long-term exposure profiles simulated by the FOCUS scenarios and models. Such a reality check certainly needs more attention for dryer Mediterranean areas.

In specially designed micro-/mesocosm experiments insight can be obtained in important fate processes of pesticides under Mediterranean conditions. However, to simulate South European exposure conditions in micro-/mesocosm experiments conducted for regulatory purposes, the adopted exposure regime should be guided by exposure predictions (e.g., on basis of relevant FOCUS or national scenarios and models) and/or chemical monitoring data from these systems. To inform the experimental design of micro-/mesocosm experiments for regulatory purposes, typical but generalized exposure profiles need to be derived that provide information on peak concentration, pulse duration, interval between pulses, background concentration, and the area under the curve concentration between time points (Brock et al. 2010).

In the prospective risk assessment procedure for placing pesticides on the European market, usually individual active ingredients are tested. However, it can be argued that a realistic impact assessment also requires the testing of mixture toxicity and multiple stress caused by the use of different active ingredients in the same crop or in different crops grown in the same watershed. Adopting the crop approach and expressing the dynamics in pesticide exposure concentrations in terms of toxic units might be a promising way forward (see, e.g., Van Wijngaarden et al. 2004; Arts et al. 2006) to address multi-stress of pesticide exposure under typical Mediterranean cropping conditions.

#### 4 Direct and indirect effects

Although it is reported that toxicity may be higher at higher temperatures (e.g., Mayer and Ellersieck 1986), it should be noted that also field dissipation rates of pesticides may increase with higher temperatures, herewith leading to shorter exposure times. According to Maltby et al. (2005), the distribution of species sensitivities of aquatic arthropods to insecticides does not vary markedly between climatic zones, biogeographical regions, and habitat types. These observations may also explain that the threshold levels for toxic effects of chlorpyrifos in microcosm/mesocosm experiments performed in different parts of the world were very similar, at least when a similar exposure regime was studied (Van Wijngaarden et al. 2005b; Brock et al. 2006; López-Mancisidor et al. 2008a, b; Daam et al. 2008). Tarazona (2005) discussed that no fixed sensitivity tendencies for direct effects of pesticides between different regions in the EU can be substantiated from current knowledge. However, this author also stipulated that due to differences in species composition and the role of each taxonomic group within the ecosystem, differences regarding indirect and long-term effects can be hypothesized (Tarazona 2005). In chlorpyrifos-stressed microcosms

simulating and comparing Mediterranean and temperate conditions, Van Wijngaarden et al. (2005b) demonstrated that algal blooms (indirect effects due to the decline in cladocerans) were more pronounced and persistent under Mediterranean conditions at exposure concentrations ten times higher than the threshold level of effects. In part, these observations could be confirmed in outdoor mesocosm experiments with chlorpyrifos conducted in Spain (López-Mancisidor et al. 2008a, b), although the algal blooms were less pronounced in this study.

It appears from the above that the spatio-temporal extrapolation of threshold levels for direct effect of pesticides probably is possible with relatively low uncertainty in contrast to the nature and extent of indirect effects. If, from a regulatory point of view, short-term effects of pesticides are acceptable, the factors that govern the nature, extent, and persistence of possible indirect effects need further attention when conducting and interpreting micro-/mesocosm experiments in the South zone of Europe.

#### 5 Recovery potential

Van Wijngaarden et al. (2005b; indoor) and López-Mancisidor et al. (2008a, b; outdoor) reported a greater time-to-recovery of cladoceran populations affected by chlorpyrifos under Mediterranean compared to temperate conditions. Explanations reported for this in these studies are (1) high temperature at the time of the study compared to optimal temperature for *Daphnia*, (2) increase in digestion-resistant phytoplankton species, and (3) occurrence of a dense (toxic) algae blooms (Van Wijngaarden et al. 2005b; López-Mancisidor et al. 2008a, b). This implies that the post-treatment sampling period of the microcosm studies will have to be sufficiently long as to enable demonstration of recovery of affected populations and/or communities. Interestingly, Mediterranean macroinvertebrate taxa have been reported to have a remarkable capability to recover from disturbance in comparison with non-Mediterranean taxa due to greater dispersion and (re) colonization capabilities (Bonada et al. 2007).

Given the low availability of semi-field studies in the South zone, the evaluation of the recovery potential of sensitive freshwater populations in micro-/mesocosm experiments conducted in the South zone will provide valuable new insights on how to extrapolate the No Observed Ecologically Adverse Effect Concentration of these test systems to derive a RAC.

#### 6 Conclusions

Although direct effects of chlorpyrifos appeared similar in Mediterranean and temperate model ecosystem studies,

exposure profiles, indirect effects, and recovery potential may be different between these regions. In addition, a larger range of compounds needs to be tested under Mediterranean conditions as to evaluate if and to what extent results from model ecosystem studies may be extrapolated between these climatic zones. The research project in Lisbon was initiated to contribute to this by considering the discussed issues in this paper, which includes (1) ecological scenarios (to compare communities and effects between microcosms and field), (2) exposure (FOCUS simulated exposure profiles; crop-based exposure regime), and (3) potential differences in effects and recovery with temperate regions (and implications for derivation of a RAC).

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