

## **Influence of organic matter on bio-availability of two pesticides and their toxicity to two soil dwelling predators**

**Jean-Pierre Jansen<sup>1</sup>, Louis Hautier<sup>1</sup>, Nicolas Mabon<sup>2</sup> & Bruno Schiffers<sup>2</sup>**

<sup>1</sup>*Ecotoxicology Laboratory, Department of Biological control and Plant genetic resources, Walloon Centre of Agricultural Research, Chemin de Liroux, 2, 5030 Gembloux, Belgium;*

<sup>2</sup>*Phytopharmacy Laboratory, Analytical Chemistry Unit, Faculté Universitaire des Sciences Agronomiques, Passage des déportés, 2, 5030 Gembloux, Belgium*

**Abstract:** In order to determine the influence of soil organic matter content on bioavailability of products applied to the soil and their side-effects on soil dwelling beneficial arthropods, a set of experiments with chlorpropham and carbosulfan as test products, *Bembidion lampros* and *Aleochara bilineata* as beneficial insects and pure sand with addition of 3%, 6% and 9% compost as substrate was carried out in the laboratory. Both beetle mortality or reduction in onion fly pupae parasitism by rove beetle and pesticide bioavailability were determined and compared. Products were tested at different rates according to standard IOBC methods. Bioavailability of pesticide residues was determined by chemical analysis by HPLC, comparing total extract of the substrate to a CaCl<sub>2</sub> aqueous extract that only extract pesticide residues that are not fixed on organic matter complex.

Results showed that toxicity was correlated to tested doses and organic content of the substrate. At the maximum recommended field rate, Chlorpropham lead to 96% mortality of *B. lampros* and 93% parasitism reduction by *A. bilineata* on pure sand. With addition of organic matter, toxicity rapidly decreased and the effects of the herbicide only reached 3% for *B. lampros* and 0% for *A. bilineata* with sand + 9% of compost. Similar results were obtained with carbosulfan at 1% of the recommended field rate, with 50% and 7% mortality for *B. lampros* on pure sand and on sand + 9% compost, respectively. At the same rate, 100% parasitism reduction was obtained for *A. bilineata* on pure sand, compare to 0% parasitism reduction on sand + 9% compost. Intermediate results were obtained with sand + 3% or 6% compost. Decrease in toxicity was linked to amounts of organic matter added to the sand and were indicating a strong relationship between effects, applied doses and organic matter content.

Pesticide residue analysis confirm that bioavailable doses were negatively correlated with the addition of organic matter and were strongly related to the applied dose and the organic matter content of the substrate. A comparison of dose-response relationship established on pure sand and dose-response relationship established on basis of effects obtained on sand + organic matter and bioavailable doses were indicating that the dose-response were strongly related for the 4 systems (2 products x 2 insects). These results confirm that organic matter is a major component of the soil able to immobilise pesticide residue and reduce their toxicity to beneficial organism. This propriety is discussed in regard of testing scheme for soil beneficial (selection of substrate) and in the global context of pesticide use, as the fixation of pesticide on organic matter has probably also a great impact on efficacy of products and choice of dose to be applied in the field.

**Key words:** *Bembidion lampros*, *Aleochara bilineata*, substrate, organic matter, carbosulfan, chlorpropham

### **Introduction**

In the context of a sustainable agriculture, selective pesticides are needed to control insect pest and maintain pest natural enemies populations, in order to prevent pest outbreak and secondary pest resurgence. Several studies have shown that if some foliar insecticide

applications can be selective to several beneficial arthropods (Hautier et al., 2007), most of soil applied insecticides have a great impact on natural enemies populations, especially on rove and carabid beetle that are controlling soil insect pest (Mowat & Coaker, 1967; Hassan, 1969; Edwards & Thompson, 1975; Kirknel, 1978; Finlayson, 1979; Finlayson et al., 1980; Cockfield & Potter, 1983; Floate et al., 1989; Kegel, 1989; Bale et al., 1992; Samsøe-Petersen, 1993; Hautier et al., 2007).

In some studies with different soil substrates, differences in toxicity to beneficial were observed (Hautier et al., 2006). These differences can be explained by factors as organic matter and clay content, soil moisture and temperature and microbiological activity, that can play a role in the fixation and degradation of product and, therefore, their potential toxicity to pest and beneficial arthropods (Harris, 1964, 1971, 1972; Khan, 1980; Monke et Mayo, 1990; Svett, 1991; Heimbach et al., 1992; Heise et al., 2005).

However, if the influence of these factors is known, little studies have specifically assessed one point in particular and try to make the link between bioavailability of product, representing the fraction of product that is really acting and toxic to beneficial and organic matter complex. The aim of this research was to assess the toxicity of two pesticides known to be toxic on inert substrates for beneficial, the insecticide carbosulfan and the herbicide chlorpropham, on the carabid beetle *Bembidion lampros* (Herbst) (Col.: Carabidae) and the rove beetle *Aleochara bilineata* (Gyll.) (Col.: Staphylinidae) on pure sand and on sand with addition of different amount of a standard organic matter, to specifically study the importance of this factor on the toxicity of the products, determined by classical biological test and its bioavailability, determined by pesticide residue analysis with specific extraction methods.

## Material and methods

### Substrates

Substrate used for the experiments were made of dry pure sand or dry pure sand with addition of 3%, 6% or 9% (w/w) of a standard compost used for gardening. A stock of the different mixture was prepared at the beginning of the study and kept in a dry and cool place before being used. All the substrates were analysis as there were cultural soils for texture, pH, organic content and cationic exchange capacity (CEC). Results of this analysis are presented in table 1. Percentage of sand are expressed in terms of part of the mineral fraction, not taking into account organic matter. According to Belgian database soil analysis, agricultural soils in Belgium are in the range 8-55g C organic/kg of soil (Collinet et al., 2005), with most of the time the lowest carbon contents observed for loamy-clayed soils in agricultural intensive production area. The blend of sand and 9% gardening compost reached 10g organic carbon/kg of soil (2% humus content) and was very close to these soils for this parameter. However, Cationic exchange capacity of the blend was much lower (3.1meq instead of minimum 8meq/100g for soils).

Table 1: Physicochemical characteristics of substrates used for the test: sand, organic carbon, humus content, cationic exchange capacity (CEC) and pH (\*detection limit of the method).

MO	Texture (%)			Chemical characteristics			
	Sand	Loam	Clay	org. C (g/kg)	Humus (%)	CEC (meq/100g)	pH KCl
0%	99,8	-	-	0,08*	0,16*	0,2	8,6
3%	99,7	-	-	2,5	0,5	0,9	7,2
6%	99,8	-	-	7	1,5	1,8	6,8
9%	99,8	-	-	10	2	3,1	6,4

### **Products and product application**

Carbosulfan was tested under the form of granules incorporated in the sowing line (Sheriff 1GR, carbosulfan 1%). In a 17cm-long line in the center of the exposure units, carbosulfan granules were incorporated at 1cm depth and recovered just after application. Amounts of granules were equivalent to 312.5 $\mu$ g, 625 $\mu$ g, 1250 $\mu$ g and 6250 $\mu$ g sa/m of line, corresponding to 0.5%, 1%, 2% and 10% of the recommended field rate. A 400 g/l EC formulation was used for Chlorpropham (Chlorpropham 400EC). This herbicide was tested at rates of 1200, 2400 and 4800g sa/ha in 400l/ha spray mixture, corresponding to 50%, 100% and 200% of the recommended field rate. Product was applied with the help of a laboratory sprayer, calibrated by gravimetry.

### **Bioassay with *B. lampros***

*B. lampros* adults used for the test were catch in cereal field margins with pitfall traps and small aspirator. They were kept on soils 2 to 8 weeks before being used for the tests and fed with *Ephestia kuehniella* eggs (Nutrimac<sup>®</sup>). Bioassay toxicity test with *B. lampros* were based on methods developed to test toxicity of product on the carabid beetle *Poecilus cupreus* L. in the context of registration studies at European level (Heimbach et al., 2000). Exposure units were made of a plastic box (17cmx12cmx6cm) filled with 500g of substrate. 90ml of water was added to the substrate to reach approximately 70% of the water holding capacity. 5 units of 6 beetles each were assembled for each object. Each set of experiments included 5 units of 6 beetles treated with water as control. After product application, mortality was checked at day 1, 2, 4, 7, 11 and 14. Final mortality was recorded at day 14 and corrected with control values according to Abbott formula (Abbott, 1925). The experiments were conducted in the laboratory at 20  $\pm$  2°C and 60-90% RH. The light was provided by a sodium lamp on basis of a 16:8 L/D photoperiod, with 1000-2000 lux. The validity criteria of the study was control mortality < 10%.

### **Bioassay with *A. bilineata***

Bioassay toxicity test with *A. bilineata* were based on validated methods developed in the context of registration studies at European level (Grimm et al., 2000). Insects were provided by a commercial supplier (De Groene Vlieg, Netherlands). All experiments were conducted in the laboratory. Exposure units were made of a plastic box (17cmx12cmx6cm) filled with 500g of substrate. 90ml of water was added to the substrate to reach at approximately 70% of the water holding capacity. After product application, 20 adults of rove beetles were released in each unit. There were 4 units per objects and 4 units treated with water as control for each set of experiments. Rove beetles were fed ad lib with mosquito frozen flies. 7, 14 and 21 days after product application, 500 onion fly pupae were softly introduced in each unit. The experiments were conducted in the same conditions than for *B. lampros*. Units were dismantled at day 28 and onion fly pupae softly harvested. They were kept in Berlese units to assess rove beetle emergence. The effect of the products was calculated by comparing onion fly pupae parasitism in the treated group to the corresponding control. The validity criteria of the study was parasitism rate > 30% in the control group.

### **Chemical analysis and bioavailability**

In parallel of the biological observations, samples of substrates from the units used for the toxicity tests were analysis 48h after product application. Pesticide residue was extract with two different methods: a total extraction with classical methods (acetonitrile) on the half of the sample and an aqueous CaCl<sub>2</sub> solution extraction to only extract pesticide fraction that

was bioavailable. Both extracts were thus purified with a florisil column and dosed by HPLC. Bio-availability was expressed by the ratio bioavailable residue/total residue of the same object (substrate x dose).

With both toxicity test and bioavailable pesticide residue, dose-response relationship was determined with a Probit analysis (Minitab software 13.20). Different distribution (normal, log normal and Weibull) were tested and the distribution with the best adequation coefficient was retained.

## Results and discussion

### *Bioassays with A. bilineata and B. lampros*

Results of toxicity tests with carbosulfan and *A. bilineata* on the different substrates are illustrated in figure 1. If 100% onion fly parasitism reduction was obtained for all substrates at a rate of 6250 $\mu\text{g}$  sa/m sowing line, lower tested rates gave intermediate results, with a decrease in parasitism reduction linked to the reduction of tested rate and the increase in organic content of the substrate. The decline in toxicity according to the tested rate was observed for all substrates but was faster with 6% and 9% of addition of gardening compost to the sand.

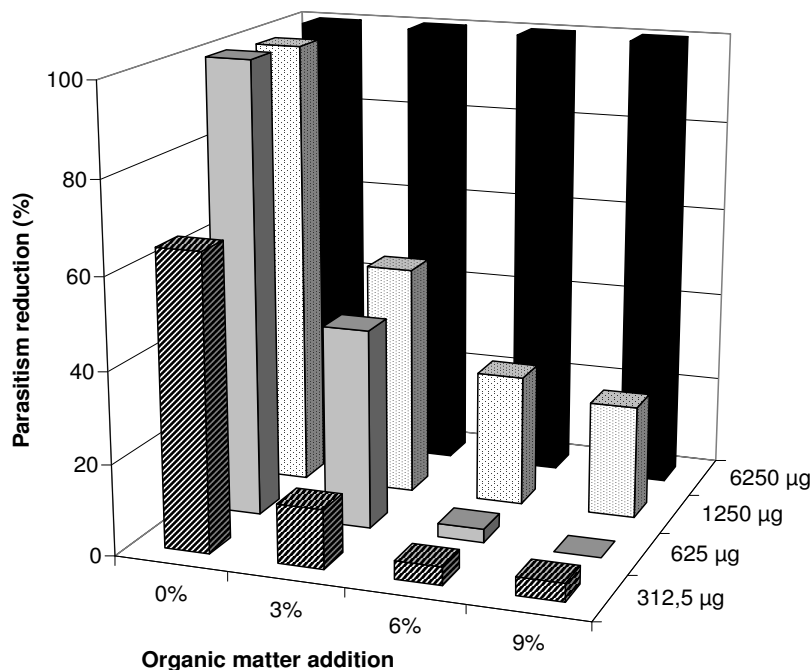


Figure 1: Toxicity of carbosulfan to *A. bilineata* on sand with addition of organic matter. Tested rate =  $\mu\text{g}$  sa/m of sowing line (field rate = 62500  $\mu\text{g}$  sa/m).

Results of toxicity tests with carbosulfan and *B. lampros* on the different substrate are illustrated in figure 2. The same trends than for *A. bilineata* were observed, with a decrease in toxicity according to the decrease of the tested rate and the increase of organic matter addition to the sand. However, compared to *A. bilineata*, *B. lampros* seemed to be less sensitive to carbofuran, with e.g. 46% and 75% effects at 6250 $\mu\text{g}/\text{m}$  sowing line on sand + 9% and 6% organic matter, compare to 100% effects for *A. bilineata* at the same rate and on the same substrates.

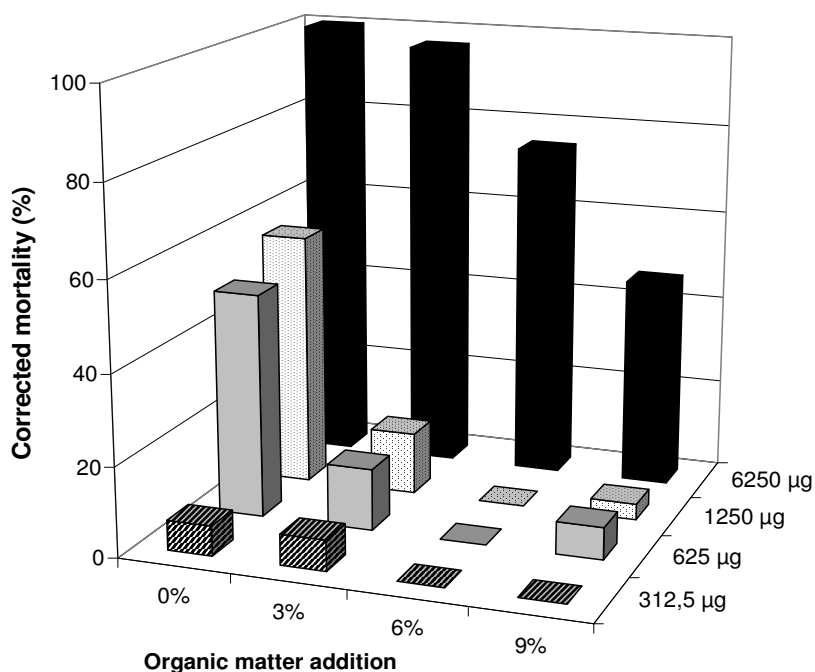


Figure 2: Toxicity of carbosulfan to *B. lampros* on sand with addition of organic matter. Tested rate =  $\mu\text{g sa/m}$  of sowing line (field rate =  $62500 \mu\text{g sa/m}$ ).

Results of toxicity tests with chlorpropham and *A. bilineata* on the different substrates are illustrated in figure 3. As for carbosulfan, the effects decreased with the increase of organic matter added to the sand and the decrease of the tested rate.

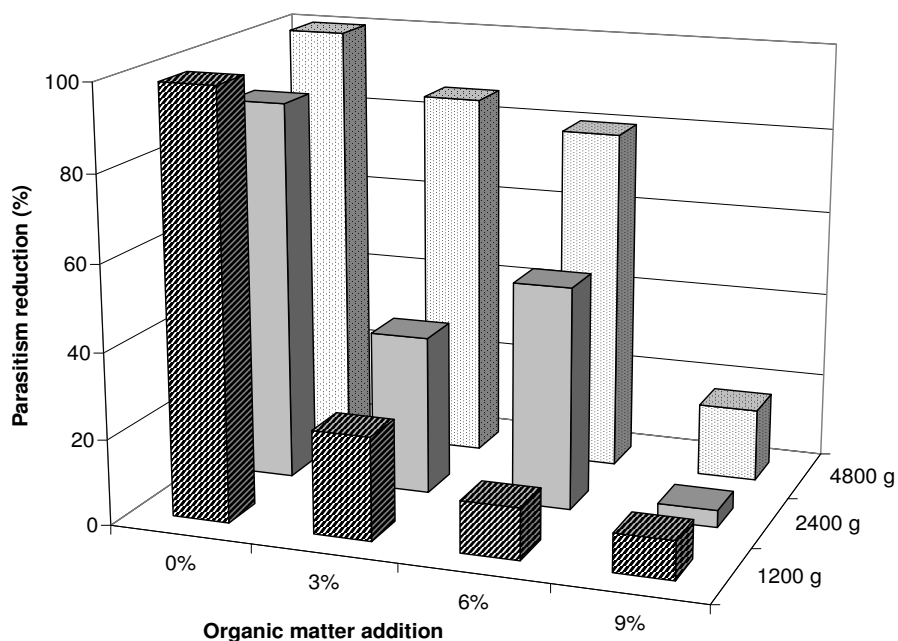


Figure 3: Toxicity of chlorpropham to *A. bilineata* on sand with addition of organic matter. Tested rate =  $\text{g sa/ha}$  (field rate =  $2400 \text{g sa/ha}$ ).

Results of toxicity tests with chlorpropham and *B. lampros* on the different substrate are illustrated in figure 4. The same trends than for other combinations product-beneficial was observed, with a clear decrease of the toxicity when organic matter was added to the sand. As for carbosulfan, *B. lampros* seemed to be less sensitive to chlorpropham than *A. bilineata* with e.g. nearly no mortality for *B. lampros* at 1200g and 2400g sa/ha on sand + 3%, 6% or 9% organic matter addition, compare to effects in the range 4-52% for *A. bilineata* at the same rates and on the same substrates.

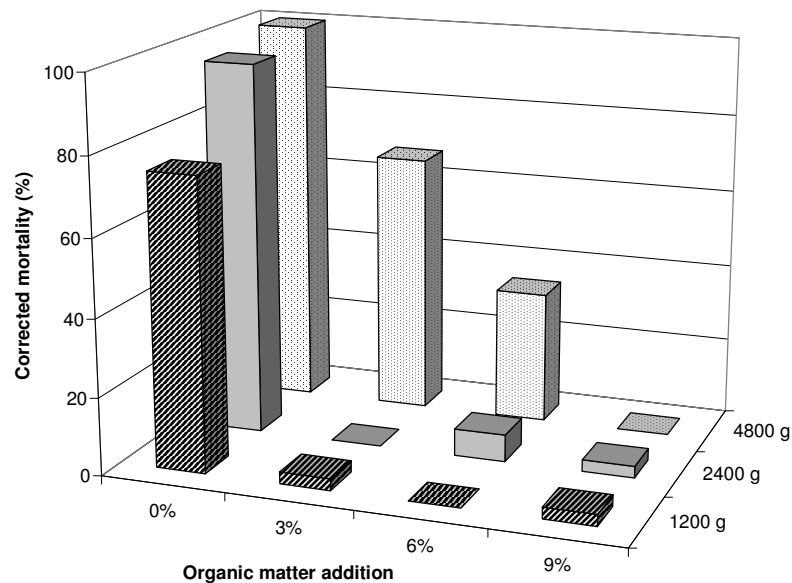


Figure 4: Toxicity of chlorpropham to *B. lampros* on sand with addition of organic matter. Tested rate = g sa/ha (field rate = 2400g sa/ha).

#### *Bioavailability of products and LR50*

With total extraction methods, a mean of 97.7% (n= 22, sd = 3.8%, min-max = 90.4-108.1%) of the expected dose applied was recovered for carbosulfan and 99.0% (n=33, sd = 3.8%, min-max = 95.8-109.2%) for chlorpropham.

Determination of bioavailability of carbosulfan and chlorpropham determined by chemical analysis on the different substrates are given in figure 5 and 6. Results showed that the bioavailability was nearly 100% on pure sand and decreased according to the increase in organic matter of the substrate. With only 3% of gardening compost added to the sand, the bioavailability of both products only reached 20% of the dose applied. With 9% of gardening compost (substrates similar in terms of organic content to agricultural soils with low humus content), bioavailability of both products was below 10% of the dose applied. In the set of concentrations tested, bioavailability was not depending of the dose applied, indicating that the relationship between dose applied and dose fixed by the organic matter was linear and no saturation of the organic matter complex occurred.

Assessment of LC50 – bioassay, based of experiments carried out on pure sand (tested rate x effect) and LC50 – bioavailability, based on experiments carried out on substrates with organic matter (bioavailable doses determined by chemical analysis x effect) are given in table 2. Comparison of both values for each system species x product was indicating that LR50 obtained by the two methods were close each other, with ratio in the range of 0.41-1.61.

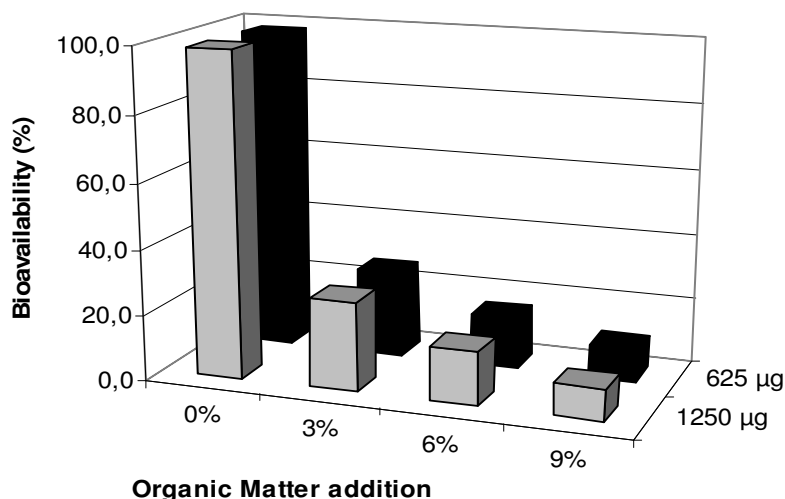


Fig. 5: Bioavailability of carbosulfan on sand enriched with organic matter, expressed in % of the dose applied.

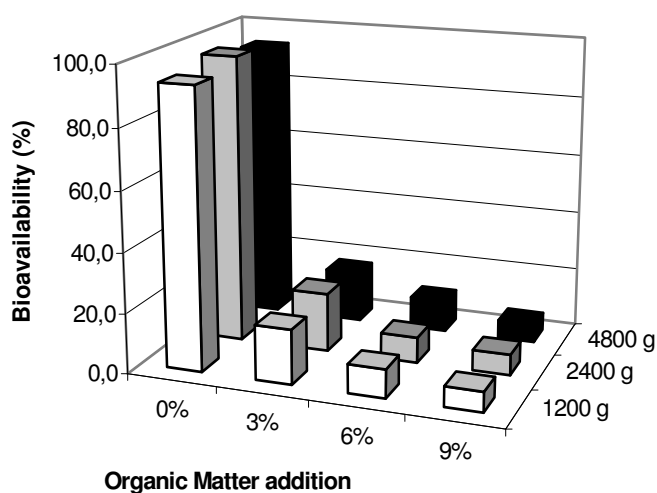


Fig. 6: Bioavailability of chlorpropham on sand enriched with organic matter, expressed in % of the dose applied.

Table 2: LC50 of products for two beneficial arthropods. Bioassay LC50 determined on basis of experiments carried out on pure sand (applied rate x effect) and Bioavailability LC50 determined on basis of experiments carried out with sand enriched with organic matter (rate determined by chemical analysis x effect).

Product	Species	Data origin	LC50 ± sd
Carbosulfan	<i>A. bilineata</i>	bioassay (sand)	167 ± 2.4 µg ai/m
		bioavailability (sand + organic matter)	269 ± 3.8 µg ai/m
	<i>B. lampros</i>	bioassay (sand)	1417 ± 178 µg ai/m
		bioavailability (sand + organic matter)	936 ± 134 µg ai/m
Chlorpropham	<i>A. bilineata</i>	bioassay (sand)	1079 ± 14 g sa/ha
		bioavailability (sand + organic matter)	449 ± 3.5 g/ha
	<i>B. lampros</i>	bioassay (pure sand)	1155 ± 101 g sa/ha
		bioavailability (sand + organic matter)	836 ± 57 g sa/ha

## Discussion

Results of the study clearly showed that the organic matter reduce the toxicity of soil applied pesticides on beneficial organisms. A simple addition of 9% (w/w) gardening compost to sand, to reach similar concentration in organic content than poor agricultural soils in Belgium for this parameter, reduce the toxicity of carbosulfan and chlorpropham for two ground dwelling predators, *A. bilineata* and *B. lampros*. At rates that previously gave 95-100% mortality on pure sand, e.g. carbosulfan at 625 $\mu$ g ai/m of sowing line for *A. bilineata* and chlorpropham at 2400g sa/ha for both species, effects were close to 0% on sand + 9% gardening compost. These results are similar than those obtained with dimethoate for *P. cupreus* larvae (Heise et al., 2005). The results of chemical analysis and bioavailability assessment of chlorpropham and carbosulfan showed that the reduction in toxicity was correlated with a reduction of bioavailability of products due to the addition of organic matter to the sand. In our conditions, with the doses and substrates tested, this relationship was linear, indicating that the fixation of pesticides by the organic matter was progressive according to the rate of both pesticide and organic matter. The dose-response relationship established on pure sand and those originating from chemical analysis and toxicity results on sand + organic matter were similar, except a small gap that can probably be solved by an improvement of the aqueous extraction method.

If results of bioavailability on pure sand also confirm that this substrate can be considered as inert with pesticides, it also demonstrate that if the field rate is tested on sand in the laboratory, the exposure of beneficial insects as carabid and rove beetles can be highly overestimated compare to the field, as a great part of the product will be immobilized by organic matter complex and not be biologically active. With carbosulfan and chlorpropham, the overestimation factor was at least 10x as less that 10% of the dose applied was available on an artificial substrate made of sand and 9% of gardening compost, with an organic content close to agricultural soils with low carbon content. This factor take only into consideration the impact of the organic content and is probably greater on natural soils that content clays, as these components are also able to fix pesticides (Schiavon & Barriuso, 2005). Thus, in the context of Tier II studies on natural substrate, semi-field and field studies for soil dwelling arthropods, composition and characteristics of the substrate used for the test is of primary importance and several products that are harmless or harmful in one substrate could be the opposite on another one.

A convenient way to make the laboratory tests closer to field conditions and real exposure of beneficial could be to determine on representative soils the bioavailability of tested pesticides and report these rates on artificial substrates in the laboratory, e.g. with the help of previously established dose-response study. However, for a more accurate approach of this system, impact of other soils components on pesticide bioavailability, as cationic exchange capacity of clays, and compoment of a broader range of pesticides must also be determined before validation of the system. It must be noted that if a huge work has been completed to study the influence of these soils parameters on pesticide mobility to develop models for groundwater protection, very little is know for beneficial arthropods toxicity.

If the organic matter can fixed a part of the pesticide and reduce risk for beneficial, it must also logically have a direct impact on the efficacy of the product. According to application of 91/414/EEC directive for registration of products at European level, the recommended field rate for a specific use is the lowest rate that is still effective. This rate is normally based on a set of efficacy trials performed in different conditions to cover all possible situations encountered when the product will be on the market. For herbicides, it is well known that fixation of the active ingredient by the clay-organic matter complex can



modify the dose that is really active. Therefore, for several products, the recommended rate is adapted to the soil characteristics, with an increase of the rate on heavy soils with high clay and/or organic matter content and a reduction of the rate for sandy soils with low clay and/or organic matter concentrations. The main reason of this is that if the bioavailable rate applied for herbicide is too low (e.g. a great part of the product is fixed on organic matter), the efficacy of the product will not be sufficient. And if the bioavailable rate is too high, selectivity of the herbicide is reduced and crop damages can be observed, with direct yield lost. For insecticides, the field rate is fixed for all soils and is generally based on efficacy trials realized in the worst-case pedological conditions, on heavy soils with high organic matter and/or clay contents. By this way, field rate that is still effective on these soils are effective on all soils. The direct consequence of this is that for in other soil than the worst-case study, rates applied are most of the time overestimated, with an increase of toxicity for beneficials that could be avoided if the dose was adapted to the soil. Our results have shown that an increase or a decrease of less than 1% of organic matter of the soil, e.g. between results obtained on pure sand (0% Humus) and those on sand + 3% gardening compost (0.5% Humus) can modify bioavailability of products and their toxicity for beneficial insects. Thus, if the overestimation of the field rates has no impact on efficacy of the product, as the product is applied in excess, the main problem is the toxicity of the insecticides applied to the soils for beneficials arthropods.

Most soil applied insecticides are broad spectrum products and are highly toxic at field rate for soil dwelling predators (Mowat & Coaker, 1967; Hassan, 1969; Edwards & Thompson, 1975; Finlayson, 1979; Finlayson *et al.*, 1980; Kirknel, 1978; Cockfield & Potter, 1983; Vickerman *et al.*, 1987; Floate *et al.*, 1989; Kegel, 1989; Casteels and De Clerq, 1990; Bale *et al.*, 1992; Samsoe-Petersen, 1993; Sivasubramanian & Wratten, 1995; Hautier *et al.*, 2007; Jansen *et al.*, 2008). For these products, a reduction of the recommended rate can probably limit negative impact on beneficial organisms. The way of thinking is to apply the products in term of bioavailable recommended rate, depending on the soils characteristics and the dose applied, instead of a fixed rate, leading most of the time to an overestimated rate in terms of efficacy. In the context of sustainable agriculture and pesticide risk reduction program, this decrease is welcome to limit the impact of pesticides on human health and environment and improve IPM programs. This is of particular importance for soil applied insecticides that are highly criticized for these factors and will probably be not included in the Annex I of the European registration list.

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