Comm. Appl. Biol. Sci, Ghent University, 72/2, 2007

INFLUENCE OF ORGANIC MATTER ON BIO-AVAILABILITY OF CARBOSULFAN AND ITS TOXICITY ON A CARABID BEETLE

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SUMMARY

Study of factors influencing soil insecticide toxicity are needed to reduce negative impacts of these products on beneficial insects. To date, if high toxicity differences between different type of soils have been reported, there is few specific studies on soil parameters influence on selectivity of soil insecticides to beneficial arthropods. To assess the specific impact of organic matter, the relationship between bio-availability of a soil insecticide, carbosulfan [Sheriff 1 Gr], and its toxicity on a small Carabidae, *Bembidion lampros* (Herbst.) on a sand enriched with increasing quantities of organic matter was studied.

In laboratory, adults of *B. lampros* were put on different substrate, made of sand or sand with addition of organic matter at 3, 6 and 9% w/w, and treated with carbosulfan applied as granule at the rate of 312.5, 625, 1250 and 6250 µg a.i./m corresponding respectively to 0.5, 1, 2 and 10% of the recommended field rate. Mortalities of *B. lampros* were assessed after 14 day of exposure. In parallel, the total carbosulfan residue (total extraction) and bioavailable fraction ($CaCl_2$ aqueous extraction) were determined 48h after substrate treatments. According to the mortalities and bio-availability obtained, a dose - response relationship was calculated and compared with a reference relation dose - response obtained on sand, where the bio-availability of the product was considered as 100% of the amount of product applied.

Carbosulfan was highly toxic on sand for *B. lampros*, with 100, 57 and 50% mortality at 10, 2 and 1% of the recommended field rate. When organic matter was added to the sand, the toxicity gradually decreased. This reduction in toxicity was more rapidly observed on sand + organic matter than on pure sand. The mortalities were strongly correlated with the bio-availability, indicating first that the organic matter is fixing an important part of the insecticide and secondly reduce its toxicity to beneficial arthropods. The results suggest that it could be possible, with further research, to predict toxicity of products in the field on different kind of soil according previous laboratory toxicity models and soil analysis.

In conclusion, the organic matter influences strongly bio-availability of carbosulfan. This bio-availability was strongly correlated to toxicity to *B. lampros*. With the improvement of bio-availability determination and method validation, the assessment of pesticide bio-availability in the substrate could help to estimate the pesticide toxicity towards carabidae on different type of soils.

INTRODUCTION

In the context of a sustainable agriculture, selective pesticides are needed to both 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 and Coaker, 1967; Hassan, 1969; Edwards and Thompson, 1975; Kirknel, 1978; Finlayson, 1979; Finlayson *et al.*, 1980; Cockfield and Potter, 1983; Floate *et al.*,1989; Kegel, 1989; Bale *et al.*, 1992; Samsoe-Petersen, 1993; Hautier *et al.*, 2007).

In some studies with different soil substrate, differences in toxicity to beneficial were observed (Hautier *et al.*, 2006). These differences can be explained by several points, as organic matter and clay content, soil moisture and temperature and microbiological activity, that can play a rule in the fixation and degradation of product and, therefore, their potential toxicity to pest and beneficial arthropods (Harris, 1964, 1971, 1972; Khan, 1980; Monke and Mayo, 1990; Svett, 1991; Heimbach *et al.*, 1992; Heise *et al.*, 2005).
However, if the influence of these factors are known, little studies have specifically tried to assess one point in particular and try to make the link between bioavailability of product, that is representing the fraction of product that is really acting as insecticide and toxicity to beneficial. The aim of this research was to assess the toxicity of one insecticide known to be toxic for beneficial, carbosulfan, on the carabid beetle *B. lampros* on pure sand and on sand enriched with different amount of a standard organic matter, to specifically study the importance of this factor on the toxicity of the insecticide, determined by classical biological test and its bioavailability, determined by pesticide residue analysis with specific extraction methods.

MATERIALS AND METHODS

Bioassay toxicity test was 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). All experiments were conducted in the laboratory. Exposure units were made of a plastic box (17x12x6 cm) filled with 500 g of substrate. Substrate was made of pure sand or sand with addition of 3, 6, 9% (w/w) standard compost. 90ml of water was added to the substrate to humidify them at approximately 70% of their water holding capacity. The experiments were conducted at 20±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.
B. lampros adults were catched in cereal field margin 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®).
All the substrates were analysis as there were cultural soils for texture, pH, organic content and Cationic exchange capacity (CEC). Results of this analysis is presented in table 1. In a 17 cm long line in the centre of the exposure units, carbosulfan granules were incorporated at 1 cm depth and recovered just after application. Amounts of granules were equivalent to 0.3125, 0.625, 1.25 and 6.25 µg sa/m of line, corresponding to 0.5, 1, 2 and 10% of the field application rate. These rates were selected because on basis of a previous work a significant response was expected on pure sand. For each substrate, 5 test units were treated and 5 test units were left untreated as control. In each test unit, after product application, 6 adults of *B. lampros* were released. They were fed throughout the study with *Ephestia kuehniella* sterilised eggs (Nutrimac®). Mortality was observed at day 1, 2, 4, 7, 11 and 14 after product application. At day 14, units were dismantled and final assessment was recorded. Observed mortality were corrected with control values according to Abbot formula (Abbot, 1925). Results were rejected if control mortality exceed 10%.

Table 1. Substrate pedological characteristics

Concentration in organic matter	Texture (%)			Chemical caracteristics			
	Sand	Loam	Clay	C orga (g/kg)	Humus (%)	CEC (meq/100g)	pH KCl
0%	99,8	-	-	0,08*	0,16*	0,2	8,6
3%	99,73	-	-	0,25	0,5	0,9	7,2
6%	99,78	-	-	7	1,5	1,8	6,8
9%	99,75	-	-	10	2	3,1	6,4

In parallel of the biological observations, samples of substrate from the units used for the toxicity tests were analysis for carbosulfan content, 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 $CaCl_2$ solution extraction to only extract pesticide fraction that was bioavailable. Both extracts were thus purified with a florisil column and dosed by HPLC. Bioavailability 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 were determined with a Probit analysis (Minitab software 13.20). Different distribution were tested and the distribution with the best adequation coefficient (Weibull distribution) was retained.

RESULTS

Substrates

According to the soil analysis, organic carbon content reached 10g/kg of soil for the 9% substrate. This is more or less comparable to agricultural soil of intensive production area with low C content. According to Belgian database soil analysis, agricultural soils in Belgium are in the range 8-55 g C organic/kg of soil (Collinet *et al.*, 2005). However, CEC was much lower than these soils (3.1 meq/100 g for 9% organic content compare to minimum 8 meq/100 g for agricultural soil).

Bio-availability

Mean total carbosulfan dose, mean bioavailable dose and bioavailability, in % of total dose, according to substrate and tested dose are given in table 2. On pure sand, bioavailable dose was very close of the applied dose, with 98.1 and 98.6% of recovery. With sand and addition of organic content, biavailability of carbosulfan rapidly decreased, with around 10% on sand with 9% compost. Bioavailability clearly depend on the substrate, but not on the applied dose, for the doses tested. It must be pointed out that, for a normal agricultural soil with at least 8 g C organic/kg of soil, only 10% of the carbosulfan applied is available. For soil with a higher C content, the proportion probably could be more less.

Table 2. Bioavailability of Carbosulfan, expressed in µg a.i./m row determined by HPLC analysis on the different substrate and at 625 and 1250 µg a.i./m

Rate (a.i/m)	Organic matter content											
	0%			3%			6%			9%		
	A	B	C	A	B	C	A	B	C	A	B	C
625 µg	595,4	584,0	*98,1%*	650,6	171,7	*26,4%*	594,5	93,4	*15,7%*	612,0	61,9	*10,1%*
1250 µg	1202,4	1185,9	*98,6%*	1244,2	339,5	*27,3%*	1236,3	205,1	*16,6%*	1184,1	114,5	*9,7%*

Toxicity tests with *B. lampros*

Results of toxicity test with *B. lampros* on the different substrates are illustrated in figure 1. On pure sand, corrected mortality of *B. lampros* reached 7, 50, 57 and 100% at 312.5, 625, 1250 and 6250 µg s.a./m, respectively. At 6250 µg/m, the addition of 3%, 6% and 9% compost to the sand decreased mortality of *B. lampros*. With lower carbosulfan rates, the decrease in mortality was very quick, with no significant mortality on sand with 3% of organic matter. Control mortalities did not exceed 6%.

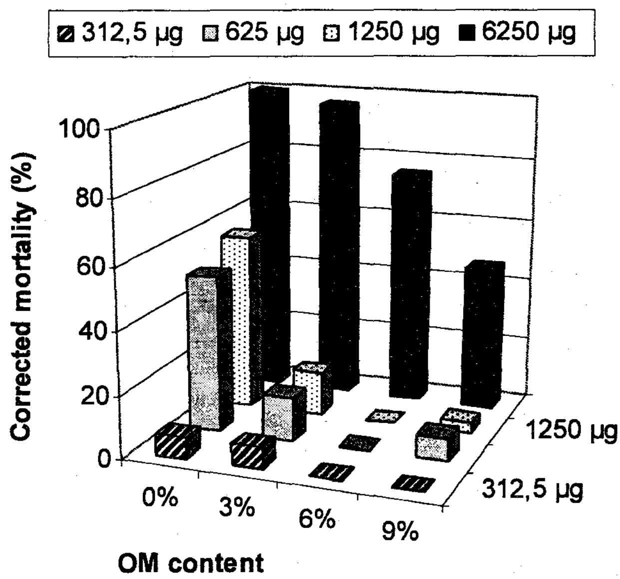

Figure 1. Corrected mortality of *B. lampros* (%) exposed to different rates of carbosulfan on sand and on sand with addition of organic matter (OM)

Dose-response relationship

Dose-response and bioavailable dose-response relationships are illustrated in figure 2. Dose response relationship was established on basis of results of test on pure sand at different rates while bioavailable dose-response was estimated on basis of mortality on sand with organic matter toxicity tests and pesticide residue dosage results. LD_{50} on pure sand reached 1417±178 µg s.a./m and bioavailable LD_{50} reached 926±134 µg s.a./m. There was a slight gap between the two methods, but both curves were very similar. Differences could came from the extraction method to determine bioavailable dose, with probably a underestimation of carbosulfan residue not fixed on organic matter.

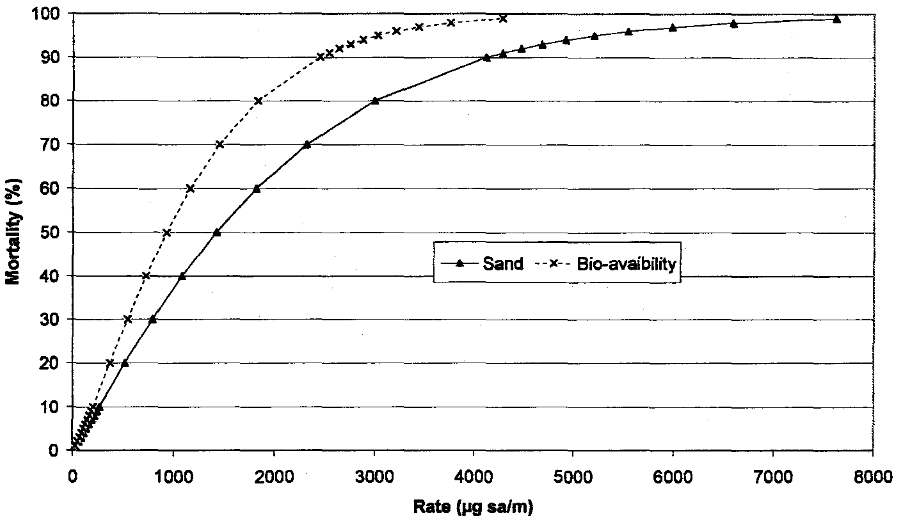

Figure 2. Dose-response relationship between mortality of *B. lampros* and carbosulfan rate on pure sand (dose applied = 100% bioavailable) and bioavailable dose on different substrates, determined by chemical analysis

DISCUSSION AND CONCLUSIONS

On pure sand, bioavailability of carbosulfan was very close to 100%, indicating that, with the uncertainty margin of pesticide residue dosage, the sand can be considered as totally inert with the pesticide. In these conditions, the insect is exposed to the maximum of the rate applied. The addition of organic matter to the sand reduce bioavailability of carbosulfan and the exact dose to which insects were exposed and, logically, toxicity of the product. In our conditions, with the tested doses and organic matter additions, this relationship was linear, indicating that the fixation of carbosulfan by the organic matter was progressive according to the rate of both pesticide and organic matter. These results suggest that it could be possible to establish dose-response relationship on sand and try to determine, with pesticide bioavailable residue analysis, possible toxicity on different soils, according to their organic matter content. However, impact of other soil components on pesticide bioavailability, as cationic exchange capacity of clays, must also be determined.

Comparison of results of bioavailability on sand and on sand with 9% of compost, that correspond more or less to a soil with a very low content in organic matter is indicating that only 10% of the dose applied is available. That could say that test on sand probably overestimate at least 10x for agricultural soil.

Carbosulfan toxicity to *B. lampros* was both influenced by tested rate and substrate organic matter content. Decrease in toxicity was very high, even with very low level of organic matter and correlated with pesticide bioavailability. In the context of sustainable agriculture, this could be a tool to try to reduce side-effects of soil insecticides. If the organic matter can fixed a part of the pesticide and reduce risk for beneficial, it could also have an impact on the efficacy of the product, the real dose acting on pests and beneficial being the bioavailable one instead of the applied one. The application rate for most soil applied insecticide is the same for all type of soil and fixed for the soil where the product is the less effective, probably a clayed soil with high level of organic content. Thus, it could be possible for other soils to reduce the rate and, in a same way, reduce the impact of insecticide on beneficial arthropods. The way of thinking is to try to adapt the dose to the soil to have a bioavailable dose sufficient to be effective against pest and adapted to soil organic matter content instead of a fixed rate per ha, probably needed for worst-case soil but overestimated for most of soils, especially soils with low level in organic content. It must be pointed out that for several herbicides, the dose is already adapted to the soil. For this category of product, if the dose is to low, the product doesn't work and the dose must be increased but if the dose is too high, the result can be a lack herbicide selectivity for the crop and yield losses, directly detected. With soil-applied insecticides, application of too much higher doses can results in a lack of selectivity of product to the beneficial arthropods, which are most of the time not detected. Thus, it could be interesting to determine, with toxicity test for beneficial and efficacy tests on soil pests, if it can be possible to reduce the dose in a certain amount to try to increase selectivity for beneficial and keep efficacy of the product. As most of soil applied insecticides are actually highly criticized for their impact on human health and environment and probably will not be included in the Annex I of the European registration list for these reasons, it could be a perspective to try to reduce the dose in most of the case and their side-effects, without loosing their efficacy. For old product as carbosulfan, chlorpyriphos and diazinon it could perhaps be too late, but with new soil applied insecticides that are actually developed, this approach can be interesting to applied the right dose, sufficient to be effective and as lower as possible to limit risk on environment and human health.

ACKNOWLEDGEMENT

This research program was funded by the Federal Public Service Food Chain Security and Environment - Directorate Animals Plants and Food and by the Ministry of the Walloon Region - Agriculture General Directorate.

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