Toxicity and possible food-chain effects of copper, dimethoate and a detergent (LAS) on a centipede (Lithobius mutabilis) and its prey (Musca domestica)

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Received 12 October 1998; received in revised form 23 March 1999; accepted 13 April 1999

Abstract

Toxicity of copper (Cu) and two biodegradable chemicals—an insecticide (dimethoate) and a detergent (LAS), was tested on a common forest litter invertebrate carnivore Lithobius mutabilis and its prey, Musca domestica. The chemicals were mixed into artificial food medium for housefly larvae, and the centipedes were kept on contaminated OECD soil and fed contaminated larvae. The concentrations of chemicals were selected to cover the whole range of survival of a prey animal: from no increased mortality (control) up to ≈100% mortality during development from egg to adult. The housefly larvae were significantly more affected than their predators by all three chemicals. The LC50 values for housefly from egg to emergence were (in mg kg⁻¹ dry mass food): Cu, 708; dimethoate, 0.330; LAS, 110. In contrast, LC50 values for L. mutabilis for all three chemicals were above the range of concentrations tested: Cu > 1500; dimethoate > 0.8; LAS > 10 000 (nominal concentrations in soil and housefly medium). No significant sublethal effects on body weight or respiration rates were found in centipedes. This may suggest that in contaminated areas, except for direct toxicity, a lack of food may be an important factor in reducing centipede populations. The centipede prey, being mostly detritivores feeding directly on and living in a contaminated medium, are more exposed to a toxicant than epigeic species such as centipedes. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ecotoxicology; Pesticides; Copper; Soil invertebrates; Predators

1. Introduction

One of the least explored ecotoxicological phenomena is the influence of toxicants on higher trophic levels through the food chain. Although a number of models have been proposed for this phenomenon, none has been successfully implemented for terrestrial ecosystems to-date. In earlier studies, it was postulated that the most important process is biomagnification of pollutants along food chains. This would mean that species occupying higher trophic levels are more endangered by intoxication than those at lower trophic levels. Such a point of view was widespread, especially in the 1960s and 1970s, and was presented in a number of ecological handbooks (e.g. Odum, 1971; Colinvaux, 1973; Collier et al., 1973). More recently, this concept has been criticised by many authors (e.g. Moriarty, 1975; Ernst and Joosse van Damme, 1983; Beyer, 1986; Van Straalen and Van Wensem, 1986;
Willamo and Nuorteva, 1987; Laskowski, 1991). It is now mostly agreed that, depending on an animal’s physiology, food preferences, trophic position and probably other as yet unrecognised factors, the intoxication of carnivores with pollutants can either increase or decrease with increasing position in a food chain (cf. Dallinger et al., 1993). This leads to the conclusion that, at least in terrestrial ecosystems, effects other than direct intoxication may be more important. Among them, one of the most interesting appears to be changes in community structure (e.g. Poulton et al., 1995; Korthals et al., 1996; Matthews et al., 1996; Abd Allah et al., 1997). In the case of predatory species, pollutants may affect food availability by reducing population size of prey animals. This situation seems especially likely in the soil/litter system, where many prey animals are more intensely exposed to toxic substances via direct contact with soil and the soil solution than surface-living predators. Indeed, two-species, prey–predator experiments on effects of toxicants on soil invertebrates have recently been advocated in a number of articles as reflecting the real-field situation better and, thus, being more appropriate for soil toxicity risk assessment (Kula et al., 1995).

In this experiment, toxicities of copper (Cu), dimethoate and linear alkylbenzenesulphonate (LAS) were tested on the common forest-litter invertebrate carnivore Lithobius mutabilis Koch. (Myriapoda) and its prey, Musca domestica L. (Diptera). The chemicals chosen represent a broad spectrum of anthropogenic pollutants, from biodegradable pesticide (dimethoate) and surfactant (LAS) to non-degradable metal (Cu). Centipedes are among the most important group of terrestrial invertebrate predators. According to Albert (1983), just two species, L. mutabilis and L. curtipes, accounted for ca. 17–27% of the total assimilation by predatory macroarthropods in the forest ecosystems studied. Thus, effects of toxicants on this group can be of particular importance for the functioning of some ecosystems.

2. Methods

The centipedes (Lithobius mutabilis) were obtained from a beech–pine forest of the Ratanica catchment, located ≈40 km south of Kraków, Poland. Samples of the whole organic layer were sieved through a 1-cm mesh sieve and the centipedes were collected from the sieved material manually after transportation to the laboratory. Before being used in the experiment, the centipedes were kept for two weeks in 30 × 50 × 20 cm² transparent plastic containers, ≈100–200 individuals in each, under constant laboratory conditions at a temperature of 15 ± 0.5°C, relative humidity ≥80%, and a light : dark regime of 16 : 8 h. The bottom of each container was covered with a layer of a moist natural soil, 2–3 cm thick, with a 2–3 cm layer of leaf litter on the surface. The animals were fed on frozen housefly pupae (Musca domestica), taken from large cultures which were maintained in the laboratory.

For toxicity tests on centipedes, adults of 15–45 mg fresh mass (10–15 mm length) were selected from the culture. Acute toxicity tests (mortality) were run on groups consisting of three males and three females kept in 16 × 11 × 6 cm³ plastic boxes, and sublethal effects (respiration rate and body weight change) were studied on animals kept separately in 11 × 7.5 × 4.5 cm³ boxes. All boxes were filled with ≈1 cm deep layers of contaminated or uncontaminated (control) standard artificial OECD soil (Løkke and Van Gestel, 1998) at 50% water-holding capacity, and covered with loose transparent plastic lids. The centipedes were fed one housefly pupa per individual, twice a week. Six replicates per treatment were used in all experiments except for ‘dimethoate-contaminated food and soil’, where only four replicates were used due to an additional test which was run with only contaminated soil (see below for details).

The medium for housefly larvae was made from 515.6 g rabbit chaw, 20 g powder milk, 10 g sugar, 0.02 g baking yeast and 1 l of distilled water or experimental solution. After mixing the ingredients, the medium was left for 2 h at room temperature before use in the experiment. The larvae were kept in 120-ml plastic containers with 77 g of medium (wet weight), covered with a filtering tissue. The boxes with the larvae were kept under the same environmental conditions as the centipedes.

In the tests on houseflies, six replicates per treatment were used, and each replicate consisted of 40 freshly emerged housefly larvae. Five days after transferring larvae to the experimental medium, a layer of paper tissue was put on the surface of the medium to provide larvae with a place suitable for pupation. This
five-day period was chosen based on earlier observations on the time required to start pupation in an uncontaminated medium. When the pupae started to turn dark-brown and no more new pupae were observed, all the pupae were removed from the paper tissue, and the remaining medium was searched for specimens that had not migrated to the surface and pupated inside the medium. All pupae were then transferred to clean plastic boxes and covered with a gauze that enabled observation of emerging adults. After all imagines emerged, they were killed by freezing and counted.

Copper was used as CuCl₂ (PPH POCh, Poland), dimethoate as a formulation of 400 g a.i. l⁻¹ (Chemínova Agro A/S, Denmark) and LAS was the Marlon A350 sodium alkylbenzenesulphonate with 70–90% mass as C₁₁ and C₁₂ alkyl chains (Hüls Danmark A/S). The chemicals were mixed into the artificial food medium for housefly larvae at the same nominal concentrations as into the OECD soil used in the centipede tests. Soil was left to equilibrate for 24 h at ca. 5°C prior to use in the experiments. The concentrations of chemicals were adjusted so that they covered the whole range of survival of a prey animal: from no increased mortality (control), to almost 100% mortality during development from egg to adult. In the ‘soil + food’ experiments, centipedes were kept on contaminated soil and fed housefly pupae originating from a medium which had been treated with similar nominal concentrations of the test chemicals. Because preliminary studies showed no increased mortality of centipedes in dimethoate treatments at the range of concentrations which housefly larvae could survive, an additional test was performed with only contaminated soil (‘dimethoate-soil’), but at higher rates than could be used in a housefly test. Insufficient numbers of centipedes forced us to reduce the number of replicates in one of the tests, so that only four replicates were used in the ‘dimethoate-food + soil’ treatment.

The following nominal treatments were used in ‘soil + food’ experiments (calculated per dry weight soil or food medium):

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Nominal Concentration (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0; 56; 167; 500; 1000; 1500</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>0; 0.012; 0.037; 0.111; 0.333; 0.800 a.i.</td>
</tr>
<tr>
<td>LAS</td>
<td>0; 16; 80; 400; 2000; 10 000</td>
</tr>
</tbody>
</table>

The nominal concentrations of dimethoate in soil used in the ‘dimethoate-soil’ experiment were: 0; 1.65; 3.10; 6.25; 12.5 and 25.0 mg kg⁻¹ (active ingredient per dry weight basis).

All experiments, with the exception of Cu, were terminated after 30-day exposure. In the case of Cu, we also measured effects after 85-days exposure to account for possible long-term effects caused by accumulation of non-degradable Cu in the bodies of centipedes fed the Cu-enriched diet.

The experimental cultures of centipedes were checked daily for mortality, and the respiration rates and body weights of individually kept specimens were recorded weekly. The respiration rate of each individual was measured in a water bath at 15°C for 1 h using constant-pressure volumetric microrespirometers (Klekowski, 1975). The animals were weighed to the nearest 0.0005 g (Precisa, Switzerland).

The LC₅₀ and EC₅₀ values for the response variables, y, were estimated using a ‘half-maximal response’ non-linear regression model (CSS-Statistics) as follows:

\[ y = b_0 - \frac{b_0}{1 + (x/EC_{50})^{b_1}}, \]

where \( b_0 \) denotes the expected response at the level of dose saturation, \( b_1 \) determines the slope of the function, and \( x \) the concentration of the chemical. After setting \( b_0 \) at 100 (100% response), there were two free parameters estimated in the regression equation: \( LC_{50} \) and \( b_1 \). The goodness of fit was determined with the least-squares method. If a hormetic effect was seen, the following model was used:

\[ y = b_0 + h x \frac{b_0}{1 + (b_0/2 + hEC_{50})/(b_0/2EC_{50})} \cdot x^{b_1}, \]

where the parameter \( h \) allows for a stimulatory effect at low concentrations (hormesis), \( b_0 \) is the response in control culture (here set to 100%), and \( b_1 \) the slope (Løkke, 1995). The calculated regressions were then plotted against nominal concentrations of chemicals in housefly medium or soil.

3. Results

The LC₅₀ values for housefly larvae for the period from egg to pupation were (in mg kg⁻¹ dry weight...
food): Cu, 689; dimethoate, 0.326; LAS, 155. With respect to the number of adults emerged, the LC50s were as follows: Cu, 708; dimethoate, 0.28; LAS, 110 (Figs. 1–3). In contrast, LC50s of L. mutabilis were higher than the highest concentrations tested for all three chemicals: Cu, LC50 > 1500; dimethoate, LC50 > 0.8; LAS, LC50 > 10000 (Figs. 1–3). No relationship between concentration and mortality was observed in the ‘food + soil’ experiment for dimethoate, while the estimated LC50 for centipedes was as high as 13.8 mg kg⁻¹ in soil contaminated with high dimethoate concentrations (Fig. 1). Thus, based on nominal concentrations, the housefly larvae were affected more than the predator by all three chemicals.

The chemicals tested differed markedly in the way they affected the survival of fly larvae and centipedes. In housefly larvae, the steepest response curve was found for dimethoate, where no increased mortality was observed up to 0.111 mg kg⁻¹, yet at 0.8 mg kg⁻¹...
no single larva survived to pupation (Fig. 1(a)). A relatively sharp decline in survivorship was also observed in the Cu-contaminated medium with no increase in mortality up to 167 mg kg\(^{-1}\), and only 25% surviving at 1500 mg kg\(^{-1}\) (Fig. 2(a)). The most shallow response curve was in the relationship of larval survival with LAS concentration: survival decreased gradually from 75% of the control at 16 mg kg\(^{-1}\), to >16% of the larvae entering the pupal stage and 5% surviving to imagines at a concentration as high as 10 000 mg kg\(^{-1}\) (Fig. 3(a)). Thus, only about a sevenfold increase in dimethoate concentration was sufficient for housefly survival to drop from 100% to 0, while for Cu a ninefold concentration increase caused only a 75% increase in mortality, and for LAS the houseflies were able to survive over at least a 10 000-fold range of concentrations.

The relationships between centipede survival and chemical concentration in the soil and housefly medium differed from those found for the housefly. For dimethoate, over the range of concentrations at which housefly larvae survived, there was no significant relationship between centipede mortality and concentration (Fig. 1(b)). This was true up to 5 mg a.i. kg\(^{-1}\).
soil, when mortality rates of the centipedes increased. However, above this concentration, the curve was very steep, so that at 25 mg a.i. kg\(^{-1}\) no single centipede survived the duration of the experiment (Fig. 1(c)). After 30 days, Cu did not affect centipedes significantly at concentrations up to 1000 mg kg\(^{-1}\), but above this concentration a very abrupt increase in mortality was noted (Fig. 2(b)). After 85 days of exposure to Cu-contaminated food and soil, the centipedes exposed to 56–1000 mg kg\(^{-1}\) survived considerably better than control animals, while the abrupt increase in mortality above 1000 mg kg\(^{-1}\) was still visible (Fig. 2(c)). For LAS, the relationship between concentration and centipede survival was unclear and, on average, only a slight decrease in survival was observed. However, at a nominal concentration of 10 000 mg kg\(^{-1}\), survival of centipedes was still as high as \(\approx\)85% of the control (Fig. 3(b)).

At the termination of the experiment, no significant sublethal effects (body weight change or respiration rates) were found in centipedes (Figs. 1–3).

4. Discussion

Our studies indicate that *L. mutabilis* is relatively resistant to all chemicals tested. All three chemicals caused much higher mortality in cultures of its prey at the respective concentrations. This suggests that in contaminated areas lack of food rather than direct poisoning may lead to disturbances in centipede populations. Also, predation pressure on resistant soil invertebrates can drastically increase when other invertebrates are affected by toxicants. This effect would be expected especially for those chemicals with the largest difference in LC\(_{50}\) between a predator and its prey.

Among the chemicals tested in our studies, the direct toxicity of LAS to centipedes appears negligible; hence, in this case exclusively secondary food-chain effects are to be expected. This result seems reasonable, as centipede prey such as fly larvae, being mostly detritivores feeding directly on and living in a contaminated medium, are more exposed to a toxicant...
than epigeic species such as centipedes. Biodegradable surfactants such as LAS exert toxic action mainly through direct contact in water solution. Thus, soil-dwelling invertebrates are exposed to direct toxicity of surfactants via the soil solution, while for surface living forms there may be practically no effect.

There was also a substantial difference between centipedes and housefly larvae in their LC_{50}s for dimethoate. As dimethoate is highly toxic, both in aqueous solution and as aerosol or vapour, the large difference in toxicity was probably not caused solely by the different life styles of housefly larvae and centipedes but also through dimethoate selective toxicity against the insects. It seems, thus, that although dimethoate is considered a broad-spectrum insecticide (Duffield and Aebischer, 1994; Duffield et al., 1996; Huusela-Veistola, 1996), it is relatively safe for centipedes. As a consequence, secondary food-chain effects may be more important with dimethoate as in the case of LAS. On the other hand, dimethoate has been found to be highly toxic to both, Coccinella and Chrysopa (Dimetry and Marei, 1992). Thus, in the case of dimethoate, the relative importance of direct toxicity and secondary community-level effects to carnivores probably depends not only on differences in life styles (endo- or epigeic) but also on the relative taxonomic positions of a predator and its prey: if a pesticide is specific enough to affect the prey population almost exclusively, then mostly secondary effects on predators can be expected. Consequently, it may be anticipated that the larger the taxonomic distance between the predator and its prey, the more important the secondary effects will be.

For Cu, the LC_{50} for centipedes was also above the range of concentrations tested and was estimated at ca. 1700 mg kg^{-1}, while for houseflies it was approximately one-third that value. This difference was, however, substantially smaller than in the case of dimethoate and LAS and suggests that, in this case, direct toxicity may be important.

Ecotoxicological studies on the effects of toxicants on terrestrial carnivorous invertebrates have followed two directions. On the one hand, a number of studies have been performed on concentrations of toxicants in animals belonging to different trophic levels in the hope of finding some ‘general rule’, such as biomagnification, which would indicate which organisms are the most endangered in contaminated ecosystems. On the other hand, probably even more research has been carried out on the side-effects of pesticides, mostly on carnivorous beetles and spiders, but also on other ‘beneficial’ arthropods. While the first approach apparently failed (cf. Beyer, 1986; Laskowski, 1991), the latter studies concentrated mostly on direct toxic effects (e.g. Buchs et al., 1989; Gilgi et al., 1996). Although the biomagnification hypothesis did not appear to be universal, other ‘food-chain’ effects seem possible. Among them, the most important from a purely ecological point of view, are changes in interactions between populations of different species exposed to toxicants (community-level effects) (cf. Cohn and MacPhail, 1996). In many cases, the most significant among these interactions are predator–prey relationships, because the abundance of prey ultimately determines the survival and fecundity of the predators. For example, Purvis and Bannon (1992) found that application of methiocarb-based slug pellets caused different effects in carabid populations, depending on the season, and attributed these differences to purely indirect effects, such as prey availability and foraging behaviour. Possible interactions between metal contamination and community-level biotic factors were studied recently by Kiffney (1996). This author found that metals can significantly influence the relative importance of biotic factors in altering freshwater macroinvertebrate community structure: in streams dosed with metals, predation intensity was greater than in controls. On the other hand, Parmelee et al. (1993) found that soil-dwelling predatory nematodes were more susceptible to Cu contamination than their prey (also nematodes), which resulted in reduced predation on herbivorous nematodes and, in consequence, greater numbers of nematodes compared to controls. Such results indicate how important these interactions are and how inaccurate a risk assessment may be if based solely on single-species, direct-toxicity tests.

The results obtained in this study, as well as those reported by Parmelee et al. (1993) and Kiffney (1996), add another dimension to laboratory-based risk assessment procedures. They suggest that, in some situations, there may be secondary community-level effects, understood here as interactions between toxic substances and biotic factors, rather than direct poisoning, which can determine the final impact of a toxicant on a number of species in a polluted...
ecosystem. The species particularly susceptible to secondary trophic-level effects would probably be the epigeic predators feeding on endogeic fauna. This leads to the conclusion that selectivity of a particular toxicant against particular species or a group of species, should only be the first step of risk assessment studies. A similar conclusion was also reached by Duffield et al. (1996), who studied recolonisation patterns of predatory invertebrates in fields sprayed with insecticides.

Acknowledgements

We are grateful to all members of the SECOFASE project for their co-operation and fruitful discussions during project workshops. Special thanks are due to Dr. Hans Løkke, the Project Co-ordinator, for all his help. We also wish to thank to Dr. Philip Heneghan for a critical reading of the manuscript. The funding for this work was provided by the EU Programme Environment 1990–1994 (the PECO Programme Contract No. ERB-CIPD-CT93-0059).

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