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Comparative Acute Toxicity of Some Pesticides, Metals, and Surfactants to *Gammarus italicus* Goedm. and *Echinogammarus tibaldii* Pink. and Stock (Crustacea: Amphipoda)

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The utilization of invertebrates as target organisms within a battery of tests in aquatic toxicity assessment has become increasingly important since it was evident that the water quality standards based only on toxicity data towards fishes would not be able to grant ecological health of aquatic ecosystems. Considering the key trophic role of Amphipoda in freshwater ecosystems, we have studied the possibility of including these organisms in such a battery. The choice was between *Gammarus italicus* Goedm. and *Echinogammarus tibaldii* Pink. & Stock, both endemic to Central Italy and Sardinia (the latter also to Sicily). The preliminary knowledge of the sensitivity of these organisms to pure chemicals was the necessary starting point for their useful utilization, both in acute and in chronic toxicity tests. With regard to freshwater Crustacea, a large toxicity data base is available only for *Daphnia magna,* which is now considered as a reference organism in aquatic toxicology. So far acute and chronic toxicity data towards freshwater Amphipoda are relatively scarce and only related to few species. The aim of this study was therefore to assess the acute toxicity of some common pesticides, metals and surfactants towards G. *italicus* and *E. tibaldii,* and the possible significant differences in sensitivity between these freshwater Amphipoda.

MATERIALS AND METHODS

Chemicals, CAS numbers and purity are listed in Table 1. Pesticides (PESTANAL®) (chemicals no. 1-18) were purchased from Riedel-de-Kaën, SDBS (dodecylbenzene-sulfonic acid sodium salt) from Sigma, Brij 30 (polyethylenglycoldodecylether) and CTABr (hexadecyltrimethylammonium bromide) from Fluka, and metals from Merck.

Organisms, collected near the spring of River Vera (L'Aquila, Italy), where they live in sympatry, and screened from major detritus, were transported to the laboratory under continuous aeration and held for acclimation in cool and aerated water in 20 L aquaria.

Mature adult male individuals obtained from precopula pairs and kept for about three days in cool "reconstituted water" (Organization for Economic Cooperation and Development 1981), received artificial oxygenation and were fed on dry poplar leaves previously soaked in spring water in order to enrich them with fungi and bacteria. Feeding was suspended 24 hr before experiments. "Reconstituted water" hardness and alkalinity were 240 mg/L and 55 mg/L as $CaCO_3$ respectively and pH was 7.9±0.5.

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After range finding tests, stock solutions of the chemicals were prepared in order to obtain six concentrations at the same common logarithmic intervals, plus the control. Reagent grade acetone used to dissolve the water insoluble pesticides was proved in preliminary experiments to be non-toxic to both test organisms by using concentrations 1000 times higher than those used in the experiments. The definitive tests were carried out by adding 20 animals (in duplicate for the more abundant and available *E. tibaldii)* in each 1 L glass jar containing 250 mL of the precooled aerated solution.

The comparatively low number of *G. italicus* employed in the tests should not significatively decrease the precision of the results (Douglas *et al.* 1986). Those animals which, after 96 hr, when gently prodded with a spatula, showed no movements of the pleopodes, were considered dead. During the test, carried out in static conditions and without aeration, the animals were not fed, and like in all handling and rearing operations, the temperature was kept at 8 ± 0.5°C. Experimental data were analysed through probits (Finney 1971) and the LC_{50} values were expressed as nominal concentration of toxic substances.

RESULTS AND DISCUSSION

For both organisms the LC_{50} values of insecticides (Table 1, chemicals no. 1-15) vary from less than 1µg/L for Azinphos methyl to some mg/L for Dimethoate. The differences in sensitivity between *G. italicus* and *E. tibaldii* seem not to be high, even though only for DDT and Dimethoate the 95% confidence intervals of LC_{50} overlap each other. In fact the 96-hr LC_{50} values ratio (*G. italicus/E. tibaldii*) ranges from about 19.5 for Chlormephos to 0.2 for Methomyl. The sensitivity to Dimethoate and Oxamil seems to be about the same for both organisms; *G. italicus* seems to be a little more sensitive to Azinphos methyl, Parathion methyl and Methomyl, whereas *E. tibaldii* to the other insecticides.

Comparing our data with those available for Amphipoda from literature, we can see that the sensitivity of *G. italicus* and *E. tibaldii* for DDT seems to be lower than that from Sanders (1969, 1972) for *G. lacustris.* Indeed, for this insecticide, Nebeker and Gauffin (1964) report for *G. lacustris* an LC_{50} value higher and similar to our value for *G. italicus.* Both *G. lacustris* and *G. .fasciatus* show a higher sensitivity to Methoxychlor (Sanders 1969, 1972) in comparison with *G. italicus;* on the other end, the former seems to be a little more sensitive with respect to *E. tibaldii,* and the latter about equally sensitive. The toxicity of Lindane towards *G. italicus* is similar to that towards other Gammaridae (Sanders 1969, 1972; Bluzat and Seuge 1979; Abel 1980) but about one order of magnitude higher than that from Green *et al.* (1986) on *G. pulex.* It is also evident the very high toxicity of this insecticide to *E. tibaldii.* For Azinphos methyl, our values are only a little higher than those for other Gammaridae (Nebeker and Gaufin 1964; Sanders 1969; Verschueren 1983), and *E. tibaldii* seems to be more resistant. The value of 96-hr LC_{50} for Carbofuran towards *G. pulex* (Matthiessen *et al.* 1995) is very close to our value to G. *italicus,* and about twofold that obtained for *E. tibaldii.* For Carbaryl, the value from Bluzat and Seuge (1979) in *G. pulex* is substantially the same as our value to *G. italicus,* and only a little lower than that from Sanders (1969) in *G. lacustris: these* data are significantly higher than those we obtained for *E. tibaldii.* Amphipods on the whole seem therefore to be generally very sensitive to insecticides (Mayer and Ellersieck 1986) and are certainly among the first organisms to die when these substances enter waterbodies for a short period of time. The sensitivity of *G. italicus* and *E. tibaldii* towards the herbicides (chemicals no. 16-18) and surfactants (chemicals no. 19-21) is of about the same order of magnitude, and not very high. Our data on Atrazine are very similar to those from Taylor *et al.* (1991) in *G. pulex* and from Macek *et al.* (1976) in *G. fasciatus.*

Table 1. - Values of 96-hr LC_{50} (mg/L) with 95% confidence intervals (95% C.I.) of test chemicals to *G. italicus* and *E. tibaldii.* CAS = Chemical Abstracts Service Registry Number.

no.	Substance	CAS	Purity %	LC_{50} (mg/L) (95% C.I.)	
				G. italicus	*E. tibaldii*
1	4,4' DDT	50-29-3	99	$7.0\ (3.5\text{-}9.5)\cdot10^{-3}$	$3.9\ (3.2\text{-}5.4)\cdot10^{-3}$
2	Methoxychlor	72-43-5	99	$5.8\ (5.1\text{-}6.7)\cdot10^{-3}$	$1.6\ (1.4\text{-}1.7)\cdot10^{-3}$
3	Lindane	58-89-9	99	$2.6\ (2.1\text{-}3.0)\cdot10^{-2}$	$5.1\ (4.0\text{-}6.3)\cdot10^{-3}$
4	Azinphos methyl	86-50-0	99	$2.4\ (2.2\text{-}2.7)\cdot10^{-4}$	$4.8\ (4.3\text{-}5.5)\cdot10^{-4}$
5	Parathion methyl	298-00-0	99	$2.9\ (2.3\text{-}4.4)\cdot10^{-3}$	$4.1\ (3.8\text{-}4.4)\cdot10^{-3}$
6	Fenamiphos	22224-92-6	97	$2.0\ (1.5\text{-}2.4)\cdot10^{-2}$	$1.1\ (1.0\text{-}1.1)\cdot10^{-2}$
7	Chlormephos	24934-91-6	95	$7.8\ (6.8\text{-}9.3)\cdot10^{-1}$	$4.0\ (3.6\text{-}4.5)\cdot10^{-2}$
8	Dimethoate	60-51-5	99	3.8 (3.1-4.4)	4.1 (3.8-4.5)
9	Carbofuran	1563-66-2	99	$1.2\ (1.1\text{-}1.3)\cdot10^{-2}$	$4.6\ (4.2\text{-}5.0)\cdot10^{-3}$
10	Oxamil	23135-22-0	99	$2.2\ (2.0\text{-}2.7)\cdot10^{-1}$	$3.0\ (2.8\text{-}3.2)\cdot10^{-1}$
11	Aldicarb	116-06-3	99	$4.2\ (3.8\text{-}4.7)\cdot10^{-1}$	$2.2\ (2.0\text{-}2.5)\cdot10^{-1}$
12	Propoxur	114-26-1	99	$5.0\ (4.4\text{-}5.8)\cdot10^{-2}$	$1.5\ (1.3\text{-}1.7)\cdot10^{-2}$
13	Bendiocarb	22781-23-3	99	$4.3\ (3.9\text{-}4.7)\cdot10^{-2}$	$1.1\ (1.0\text{-}1.3)\cdot10^{-2}$
14	Methomyl	16752-77-5	99	$4.7\ (4.3\text{-}5.3)\cdot10^{-2}$	$2.5\ (2.2\text{-}2.8)\cdot10^{-1}$
15	Carbaryl	63-25-2	99	$2.8\ (2.5\text{-}3.1)\cdot10^{-2}$	$6.5\ (5.7\text{-}7.6)\cdot10^{-3}$
16	Alachlor	15972-60-8	99	19.7 (18.7-21.5)	13.0 (12.5-13.5)
17	Atrazine	1912-24-9	99	10.1 (9.13-11.6)	3.3 (2.6-4.0)
18	Molinate	2212-24-9	99	2.2 (2.0-2.3)	1.8 (1.7-1.9)
19	SDBS	25155-30-0	80	20.5 (19.1-21.6)	16.7 (15.9-17.6)
20	Brij 30	9002-92-0	-	7.6 (7.3-8.1)	3.6 (3.4-3.8)
21	CTABr	57-09-0	98	$7.2\ (6.4\text{-}8.0)\cdot10^{-1}$	$2.4\ (2.2\text{-}2.7)\cdot10^{-1}$
22	$Cd\ Cl_2\ H_2O$	10108-64-2	98	$9.1\ (7.8\text{-}10.5)\cdot10^{-1}$	1.1 (0.75-1.4)
23	$Zn\ Cl_2$	7646-85-7	98	8.8 (7.9-9.7)	25.9 (22.3-29.9)
24	$Cu\ Cl_2\ 2H_2O$	10125-13-0	99	$1.7\ (1.5\text{-}1.8)\cdot10^{-1}$	$5.9\ (5.2\text{-}6.8)\cdot10^{-1}$
25	$Hg\ Cl_2$	7487-94-7	99	$6.7\ (5.5\text{-}8.0)\cdot10^{-1}$	$5.0\ (4.6\text{-}5.5)\cdot10^{-1}$
26	$K_2\ Cr_2\ O_7$	7778-50-9	99	4.4 (3.6-5.4)	$4.6\ (4.3\text{-}5.0)\cdot10^{-1}$

The ranking of toxicity of metals to *G. italicus* is: Zn<Cr<Cd<Hg<Cu, and to *E. tibaldii:* Zn<Cd<Cu<Hg<Cr. Water hardness being the same, *G. italicus* seems to be more sensitive to Cd, Zn, Cu; *E. tibaldii* to Hg and particularly to Cr.

Published acute toxicity data for metals to Amphipoda, mainly concerning cadmium and copper, are very hardly comparable due to the different hardness of test water. In any case *G. fossarum* and *G. pulex* seem to be the most sensitive to cadmium (Musko *et al,* 1990; Williams *et al.* 1985). Our toxicity data for copper to *E. tibaldii* are very similar to those from Stephenson (1983) on *G. pulex* obtained under about the same water hardness conditions. As Figure 1 shows, the LC_{50} values of all the substances tested for *G. italicus* and *E. tibaldii* correlate fairly well (r=0.9417). The slope of the regression line is 0.977, (95% C.I.=0.830 and 1.124) indicating that the line for a theoretical 1:1 correlation (slope=1.00) fits within the confidence intervals.

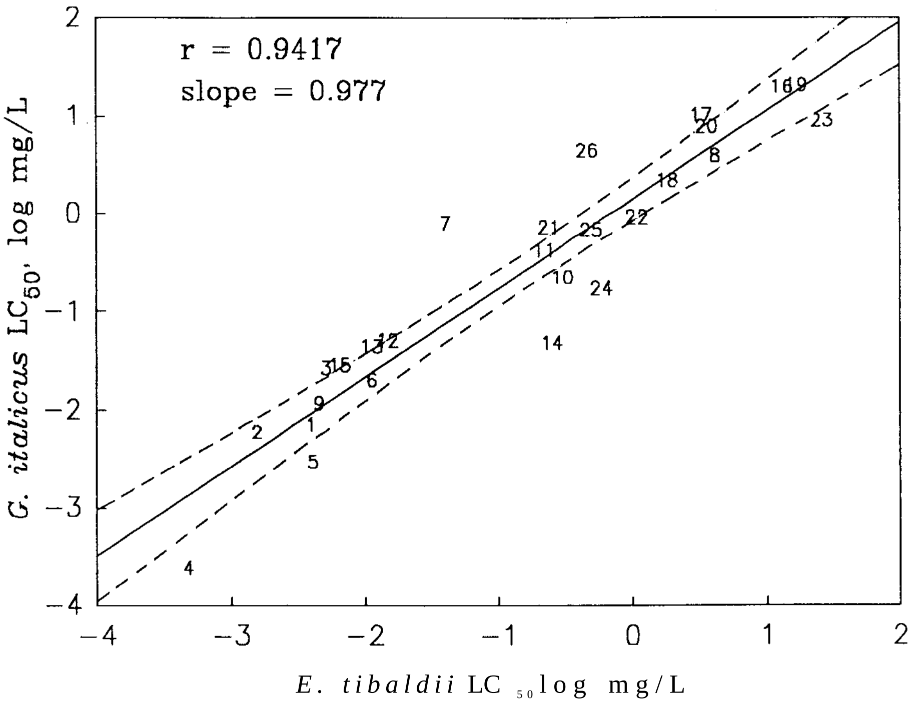

Figure 1. Comparison between LC_{50} values in *G. italicus* and *E. tibaldii.* The numbers refer to the numbering of the chemicals listed in Table 1. Dashed lines represent the 95% confidence intervals of the regression line.

The most distinct outliers from the regression line are Chlormephos (7), Methomyl (14) and potassium dichromate (26).

In conclusion, both *G. italicus* and *E. tibaldii* are potentially utilizable as test organisms because of their handling and rearing facility and of the physiological homogeneity of test individuals. Moreover they are highly sensitive towards many pollutants, particularly insecticides, and have similar responses to many tested chemicals. However, in nature, the former is present with low densities and therefore is considered an endangered species (Pavan 1982). In the light of these facts and by considering the results of the present work, we have included only *E. tibaldii* as a target organism in our toxicity test battery.

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