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# Acute Toxicity of Parathion and 2,4 D to Estuarine Adult Crabs

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Ethyl-parathion and iso-butoxyethanol ester of 2,4-dichlorophenoxyacetic acid (2,4 D) are among the most extensively used pesticides in Argentina . Their discharge to the Rio de la Plata estuary has been documented (Kuhnemann 1977; Lenardon et al. 1987). The outer section of the estuary includes the Samborombón Bay, an extensive littoral zone along the Buenos Aires Province coast. Several rivers and artificial channels flow into Samborombón Bay, the drainage of intensive agricultural runoff.

*Chasmagnatus granulata* (Grapsidae) is a species continuously distributed through the littoral zone of Samborombón Bay. *Uca uruguayensis* (Ocypodidae) was observed only on the southern edge of the Bay. All stages of the life cycle of these species are included in the aquatic trophic web, which also includes many fish species with great commercial and sport fishing value. The aims of this study were: to evaluate differences in resistance, in terms of acute lethal toxicity, to ethyl-parathion and 2,4 D : 1) between adults of *C. granulata* of two localities of the coast, and 2) between males and females of *U. uruguayensis*, a species with marked sexual dimorphism (Crane 1975).

## MATERIALS AND METHODS

*U. uruguayensis* males and females were collected in September 1986 at Faro San Antonio beach, near Punta Rasa, southern limit of Samborombón Bay (36° 18′ S and 56° 48′ W). *C. granulata* males were collected on the same date both at Faro San Antonio and the Rio Salado mouth, about 100 km northwards, in order to test differences in adults sensitivity between these areas. Crabs were acclimatized for 7 d in the laboratory at

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the conditions in which bioassays were carried out. Crabs were fed daily with chicken liver and rabbit food pellets, up to 48 hr before the beginning of assays. Weights and carapace widths of the crabs used are detailed in Table 1. Since crabs were randomly assigned to different treatments, only the dilution control individuals were weighted and measured.

Pesticide residues in crabs collected from natural populations were analyzed by gas cromatography of hepatopancreas (samples of 5 crabs). The procedure outlined in Faubert Maunder (1964) was followed to obtain purified extracts. The initial extraction with hexane was followed by a dimethylformamide partition process for fat clean-up, and a final separation of the hexane phase was done by shaking with 2 % aqueous sodium sulphate. Recovery percentages were higher than 95 % . GC conditions and equipment were: Hewlett-Packard HP5840A gas chromatographer; column: glass tube, 2 mm (inner diameter) by 4-ft, packed with 2 % OV-101 coated on Gas Chrom-W AW DMCS, 60-80 mesh; carrier gas: nitrogen at 25 mL/min; column temperature: 190°C; hydrogen flame ionized detector at 300 °C.

The acute toxicity of parathion and 2,4 D was evaluated by standard 96-h static bioassay procedures outlined by the American Public Health Association (1976). Some minor modifications of these procedures were introduced and will be discussed where appropiate. Ethyl-parathion (purity 99 %, Compañia Quimica, Buenos Aires, Argentina) and iso-butoxyethanol ester of 2,4 D (purity 95 %, Sintesis Quimica, Buenos Aires, Argentina) were employed. Pentaethylene oxide nonyl phenolate was used as solvent, in equal proportion to the pesticides, to prepare the stock solutions by adding distilled water. Dilution water used both for acclimation and assays was prepared adding artificial marine salts (Instant

**Table 1**. Mean weight (g) and mean carapace width (mm) of crabs used.

| Species / Location-Sex | Mean weight ± SD | Mean carapace width ± SD | N |
|---|---|---|---|
| *C. granulata* | | | |
| Punta Rasa-males | 17.71 ± 2.52 | 29.97 ± 1.38 | 19 |
| Río Salado-males | 19.11 ± 3.78 | 30.65 ± 1.97 | 19 |
| *U. uruguayensis* | | | |
| Punta Rasa-males | 0.76 ± 0.33 | 10.23 ± 1.53 | 20 |
| Punta Rasa-females | 0.45 ± 0.16 | 10.31 ± 1.04 | 20 |

Ocean$^{R}$) to dechlorinated tap water (80 mg/L total hardness as $CaCO_3$), to obtain the desired salinity of 12 ± 1 %. (12 g/L), pH 7 ± 0.5.

Experiments were carried out in a constant temperature laboratory (20 ± 1 °C). A controlled photoperiod of 10L:14D (incandescent light) was maintained. Assays were carried out in glass containers of 24-L capacity, each concentration in duplicate. Eight to ten crabs were placed in each container. Water volume was calculated to allow aerial respiration of the crabs (3 L for *C. granulata* and 150 mL for *U. uruguayensis*).

Tested concentrations of each pesticide are summarized in Table 2. Toxic ranges were determined by exploratory tests. The solvent concentration used in the highest pesticide concentration was run as solvent control in each series (Table 2). Dilution water controls were also run. Pesticide and solvent solutions, as well as dilution water, were renewed every 24 hr for parathion series and every 48 hr for 2,4 D series. Mortality was recorded every 24 hr, and dead animals were removed. The criterion of death used was absence of movement after gently touching the animals with a glass rod, confirmed by observation of cheliped laxity.

Probit analysis (Finney, 1971) was used to estimate the LC50 and its 95% confidence limits, with Abbot's correction for control mortality. To compare LC50 values, differences were considered to be significant when the higher LC50/lower LC50 ratio exceeded the corresponding critical value according to the American Public Health Association (1976).

## RESULTS AND DISCUSSION

Gas cromatography of hepatopancreas extracts did not

**Table 2.** Concentration series and total number of animals used (N).

| Species/ Pesticide | Pesticide concentrations | Solvent control concentration | N |
|---|---|---|---|
| *C. granulata* | | | |
| Parathion | 0.25-0.5-1-2-4-8 mg/L | 8.40 uL/L | 320 |
| 2,4 D | 1000-1580-2510-3980-6310-10000 mg/L | 8.10 mL/L | 144 |
| *U. uruguayensis* | | | |
| Parathion | 4-8-16-32-64-128-256 ug/L | 0.60 uL/L | 180 |
| 2,4 D | 12.5-25-50-100-200-400 mg/L | 0.32 mL/L | 136 |

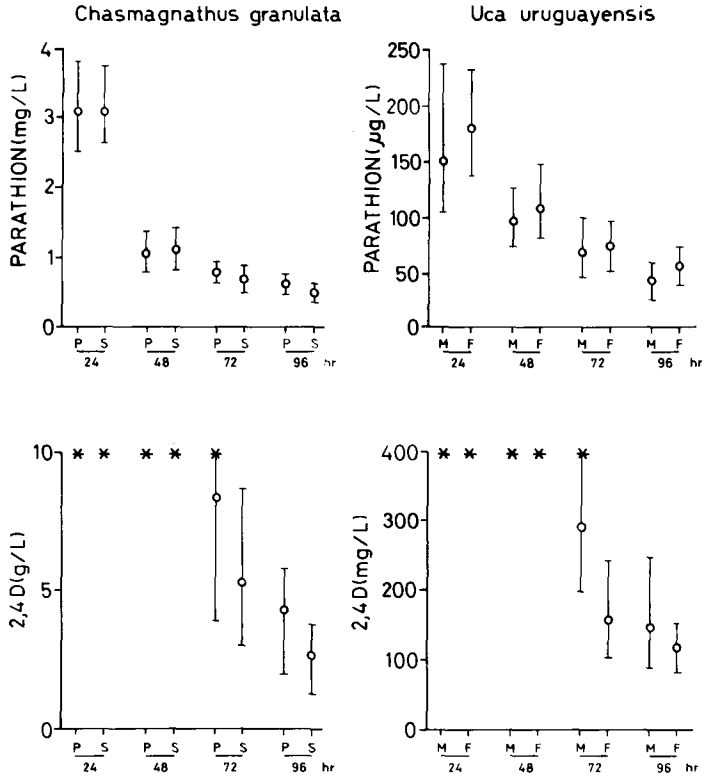


**Figure 1**. LC50 and 95 % confidence limits for both species and pesticides. *: values out of the tested concentration range. P: Punta Rasa, S: Río Salado, M: males, F: females.

show pesticide residues in crabs collected (detection limits: 1 ug/L for parathion; 50 ug/L for 2,4 D).

Student's t tests showed no differences ($p > 0.05$) in mean weight between crabs from both locations in *C. granulata*, but significant differences ($p < 0.05$) exist between sexes in *U. uruguayensis*, as expected from the sexual dimorphism mentioned.

During the acclimation period mortality was lower than 10 % . In dilution water controls, mortality was zero except for parathion-*C. granulata*, with 5 % at 96 hr. In solvent controls, mortality was also zero, except for 2,4 D-*C. granulata*, which reached 22.2 % at 96 hr, showing a relatively low toxicity of the solvent, that only affects mortality at very high concentrations (8.1 mL/L, necessary to dissolve 10,000 mg/L of 2,4 D). Sublethal symptoms observed in parathion solutions were excitation and jerky movements of the crabs, especially when gently stimulated. On the other hand,

crabs exposed to 2,4 D showed impaired mobility and loss of response to mechanical stimulus.

No significant differences were found in LC50 values between locations in *C. granulata* nor between sexes in *U. uruguayensis*, at any exposure times (Figure 1). The results of the pooled data analysis, for each species, are given in Table 3. Since different experimental conditions were used by other authors, the comparison with their results is of relative validity. Both species, *C. granulata* and *U. uruguayensis*, appear to be more resistant to 2,4 D than other aquatic invertebrates (Brooker and Edwards 1975) and more resistant to parathion than the crustaceans *Crangon crangon* and *Cardium edule* (Portmann 1972) and *Crangon septemspinosa*, *Pagurus longicarpus* and *Palaemonetes vulgaris* (Eisler 1969).

Parathion was significantly more toxic than 2,4 D for both species, about 2500-fold for *U. uruguayensis* and about 6000-fold for *C. granulata*, at 96 hr, which is consistent with results obtained for *Daphnia* (McEwen and Stephenson 1979).

*U. uruguayensis* showed a higher sensitivity than *C. granulata* to both pesticides, about 10-fold for parathion and about 25-fold for 2,4 D, at 96 hr. The absence of *U. uruguayensis* at the mouth of the Salado river is noteworthy, since this species was recorded by Boschi (1964) for this area, and could be

**Table 3**. Pooled data for each species.

| Species | Pesticide | Hours | LC50 | 95%Conf.Lim. | Slope | $R^2$ |
|---|---|---|---|---|---|---|
| *C. granulata* | | | | | | |
| | Parathion | 24 | 3.14 | (2.79-3.53) | 6.74 | 0.99 |
| | (mg/L) | 48 | 1.09 | (0.95-1.26) | 4.51 | 0.84 |
| | | 72 | 0.73 | (0.64-0.84) | 4.94 | 0.96 |
| | | 96 | 0.56 | (0.50-0.64) | 6.07 | 0.89 |
| | 2,4 D | 24 | >10 | | | |
| | (g/L) | 48 | >10 | | | |
| | | 72 | 6.73 | (5.07-9.69) | 3.57 | 0.76 |
| | | 96 | 3.37 | (2.38-4.25) | 4.21 | 0.75 |
| *U. uruguayensis* | | | | | | |
| | Parathion | 24 | 162 | (133-205) | 4.71 | 0.97 |
| | (ug/L) | 48 | 103 | ( 85-124) | 5.57 | 0.98 |
| | | 72 | 71 | ( 59- 84) | 6.91 | 0.99 |
| | | 96 | 51 | ( 42- 62) | 5.58 | 0.98 |
| | 2,4 D | 24 | >400 | | | |
| | (mg/L) | 48 | >400 | | | |
| | | 72 | 213 | (163-298) | 3.32 | 0.97 |
| | | 96 | 130 | (101-169) | 3.81 | 0.57 |

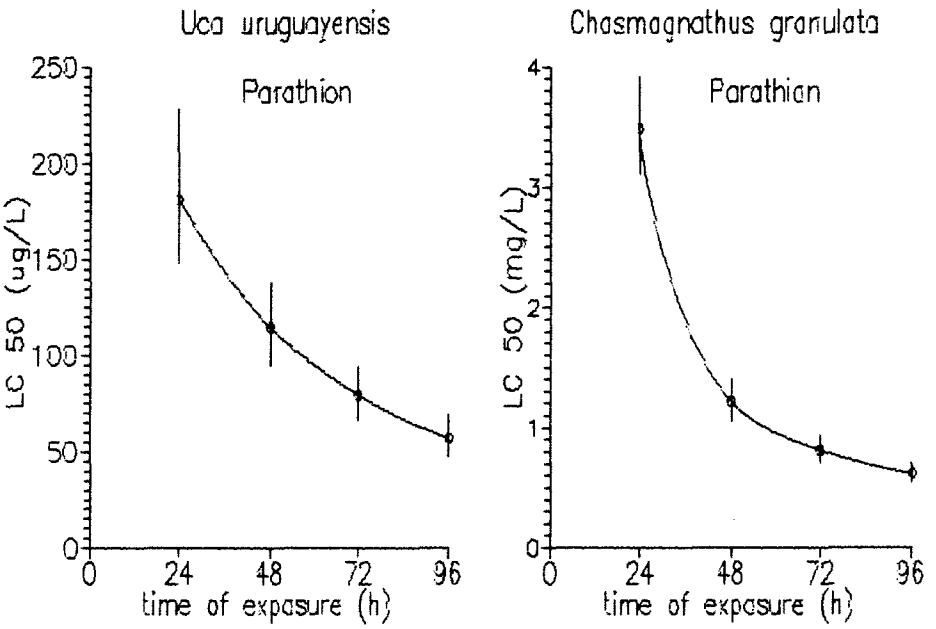


**Figure 2**. Parathion toxicity curves for each species. Bars: 95 % confidence intervals.

attributed to its lower resistance to the toxicants studied here, considering that this river discharges large amounts of pesticides into Samborombón Bay. No differences between percentages of water, exoskeleton and soft tissues of the two species were found in previous studies (Rodriguez and Dezi 1987), but the relation between LC50 values is correlated with the ratio between mean weight of both crabs, since *U. uruguayensis* is about 30 times lighter (see Table 1).

Figure 2 shows the curve of LC50 values in relation to time of exposure (toxicity curves), for assays in which four LC50 values were estimated. The asymptotic trend is evident in the parathion curves, particularly in *C. granulata*, with overlap of confidence intervals, as from 48 hr. In accordance with these results, 96-h LC50 may be considered as incipient lethal threshold concentration, for both species (American Public Health Association et al. 1976; Sprague 1969).

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