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Comparative Toxicity of Four Insecticides, including Imidacloprid and Tebufenozide, to Four Aquatic Arthropods and the Influence of Salinity on Insecticide Induced Mortality on Two Euryhaline Arthropods

Mee Young Song
Korea Research Institute of Chemical Technology
Daejon, Yusong, 305-600

During the past decade, the chemical industry has tried to develop new, more environmentally safe insecticides. Imidacloprid and tebufenozide are representatives of new insecticides that have been recently introduced. However, they have not received adequate scrutiny especially with regard to aquatic ecosystems.

Two brine-inhabiting arthropods, *Artemia* sp. and *Aedes taeniorhynchus* (Wiedemann) and two freshwater arthropods, *Daphnia magna* Straus and *Aedes aegypti* (Linn.) were selected as test species. Two crustaceans, *D. magna* (water flea) and *Artemia* (brine shrimp), were chosen as nontarget organisms and two insects, *A. aegypti* (yellow fever mosquito) and *A. taeniorhynchus* (salt marsh mosquito) were defined as target organisms.

Hypothesis in this study is that organisms inhabiting environments at or near their iso-osmotic points, are more tolerant to pollutants because there would be less osmotic stress at this balance point. Although two test brackish water organisms used have remarkable hypo-osmoregulating abilities, they would probably have less ability to deal with pollutants under hypo- or hyper-osmotic conditions. To test this hypothesis, toxicity tests were conducted under both iso- and hyper-osmotic conditions and a small flow-through system was developed to expose test species to insecticides while experiencing continuously changing salinity from hypo- to hyper-osmotic conditions and vice versa. Other objective of the present study was to compare the potential hazard of imidacloprid and tebufenozide, and two inhibitors of acetylcholine (Ach) esterase, aldicarb and dimethoate, which are both common pollutants of aquatic ecosystems, through comparative 48-h acute bioassays.

1. Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods

In this study, we determined the relative acute toxicity of test insecticides using four aquatic arthropods as model organisms. Temperature was also evaluated as a toxicity modifying factor on fresh water species to allow a general comparison with other earlier studies.

가. Experimental animals

Daphnia magna was originally obtained from Carolina Biological Supply Co. (Burlington, NC). Gravid females were placed into the culture medium (M4) developed by Elendt and Bias(1) for acclimation. Newly hatched *D. magna* (< 24 h) were used for toxicity tests. Mosquito eggs of both mosquito species were hatched in 100 mL of either M4 for *Aedes aegypti* or Artificial Sea Water (ASW, Instant Ocean® salt, 38.1 g/L Aquatic systems, Mentor, OH) for *A. taeniorhynchus* with 1.5 mg yeast at 27 °C. First instar mosquitoes (24 h old) were used for toxicity tests.

Dried cysts of *Artemia* sp. from the Great Salt Lake (Saunders Brine Shrimp Co., Ogden, UT) were hatched in a flow-through hatching system (27 °C) (2) with diluted ASW (5.6 g/L) as a hatching medium under constant illumination. Fourth stage feeding nauplii were selected for tests after 48 h incubation at 27 °C, 16L-8D.

나. Test chemicals

Tebufenozide (Mimic®), Imidacloprid (Admire®), Aldicarb (Temik®), and Dimethoate (Cygon®) were obtained or purchased from Rohm & Haas Co. (Philadelphia, PA), Miles Co. (Kansas City, MO), Rhone-Poulenc Ag. Co. (Research Triangle, NC), and Alltech Co. (San Jose, CA), respectively. All compounds were technical grade (> 95% purity) and in crystalline form. All solvents were of analytical grade.

다. Dose dependent effects

Static acute 48-h toxicity tests were conducted in basic accordance with the testing procedures of the American Society of Testing and Materials (ASTM) and the Environmental Protection Agency(3-4), except for the feeding conditions. All test organisms were fed appropriate food to avoid additional hunger stress during the test period. Four replicates with three trials were conducted. Each replicate had an appropriate solvent control plus five different concentrations of test material containing

10 animals each.

Death was determined by the lack of movement of the mouthparts for mosquitoes and the immobilization of antennae for crustaceans during 30s observations. The first observations were made after 6 h, followed by additional observations at 12, 24, and 48 h. Mortality data were corrected for mortality in the controls and subjected to probit analysis using the computer program POLO-PC(5). Slopes of probit lines among species were compared by a least square method on a log dosage-mortality scale using POLO-PC.

In general, *Artemia* sp. exhibited a greater tolerance to all test chemicals than the other species (Table 1). Imidacloprid and tebufenozide were much more toxic to mosquitoes than to the non-target crustaceans. *Aedes taeniorhynchus* was the more susceptible of the two mosquito species to all insecticides tested. This phenomenon can be easily illustrated when the maximum susceptibility ratio (LC50 most tolerant species/ LC50 most susceptible species) was calculated for each chemical based on *Artemia* sp. / *A. taeniorhynchus*. The resultant ratios were : imidacloprid (27,787); dimethoate (507.4); tebufenozide (36.9); aldicarb (36.4). Both fresh water species were more tolerant of tebufenozide at 20 °C than at 27 °C (Table 2, Fig. 1), whereas no difference in tolerance to imidacloprid between the two temperatures was observed.

Tebufenozide obviously affected the molting process and this was related to mortality in both mosquito species. As the concentration of tebufenozide was increased, a relatively larger proportion of the observed mortality was associated with the molting process (Fig. 2). Similar abnormal larval molts were observed in *Artemia* and *D. magna* exposed to tebufenozide, but no premature molting was observed in either species. Tebufenozide was also toxic to *D. magna* and *Artemia* sp., but only at concentrations far exceeding its water solubility (0.83 mg/L at 25 °C). Interruption of the molting process was associated with mortality, agreeing with the earlier study by Wing and Aller(6).

Aldicarb and dimethoate caused neurotoxic excitability in all organisms. Imidacloprid seemed to act more slowly than the two Ach esterase inhibitors inducing neurotoxicity. In both mosquito species imidacloprid caused increased mortality before or after molting with each concentration increment of the toxin tested (Fig. 2), plus it caused molt-related mortality with increasing concentrations in *A. aegypti* larvae. The two Ach esterase inhibitors caused pre-molt mortality in both mosquito species, but neither compound caused molt-related mortality in any of the four test species.

Table 1. The result of Probit analysis for each chemical on four different species at 27 °C[a].

Chemicals	N	C	Slope[b] ± S.E.	LC50 [c] (95% CI)	LC90 [c] (95% CI)
			Daphnia magna		
Imidacloprid	650	120	0.91 ± 0.14	10.44 (6.97~17.71)	263.61 (92.8~2446)
Tebufenozide	600	120	1.09 ± 0.28	17.37[d] (10.12~136)	ND
Aldicarb	690	120	1.23 ± 0.27	0.075 (0.054~0.089)	0.20 (0.14~0.15)
Dimethoate	600	120	4.70 ± 0.61	3.32 (1.73~4.12)	6.21 (5.11~10.00)
			Aedes aegypti		
Imidacloprid	600	120	4.02 ± 0.31	0.044 (0.041~0.047)	0.091 (0.082~0.11)
Tebufenozide	600	120	3.70 ± 0.50	0.92 (0.83~1.05)	2.04[d] (1.61~3.10)
Aldicarb	600	120	16.5 ± 0.13	0.29 (0.28~0.30)	0.35 (0.34~0.37)
Dimethoate	560	120	5.81 ± 0.42	5.04 (4.67~5.40)	8.37 (7.57~9.63)
			Artemia sp.		
Imidacloprid	570	120	3.47 ± 0.57	361.23(307.83~498.09)	844.81[d] (579.6~1941)
Tebufenozide	720	120	0.24 ± 0.031	5.53[d] (0.81~109.99)	ND
Aldicarb	543	120	2.77 ± 0.42	5.46 (0.86~10.20)	37.60 (16.60~660.82)
Dimethoate	585	120	1.14 ± 0.13	15.73 (8.09~34.08)	ND
			Aedes taeniorhynchus		
Imidacloprid	570	120	3.63 ± 0.55	0.013 (0.010~0.016)	0.029 (0.021~0.063)
Tebufenozide	510	105	2.81 ± 0.56	0.15 (0.11~0.20)	0.43 (0.30~1.27)
Aldicarb	600	120	4.04 ± 0.53	0.15 (0.11~0.19)	0.32 (0.26~0.46)
Dimethoate	645	90	2.36 ± 0.23	0.031 (0.023~0.041)	0.11 (0.079~0.18)

a : Column headings are abbreviated as follows : N is number of insects treated with a specific insecticide ; NC is number of test organisms treated with solvent only ; S. E. is standard error.

b : The equality tests between the slopes of two probit lines are performed as a routine of POLO-PC program. All four probit lines for the same species are different from each other ($p < 0.05$).

c : The LC values (mg/L) are given with the 95 % confidence interval in parentheses.

d : Beyond the solubility of the compound in water

ND: Could not be determined due to large heterogeneity.

Table 2. The result of Probit analysis for each chemical on the two fresh water species at 20 °C[a] .

Chemicals	N	C	Slope[b]±S.E.	LC50 [c] (95% CI)	LC90 [c] (95% CI)
			Daphnia magna		
Imidacloprid	655	120	1.86±0.17	17.36 (12.51~30.05)	85.19 (43.86~315.47)
Tebufenozide	542	120	1.94±0.83	d	d
Aldicarb	586	120	3.83±0.55	0.74 (0.67~0.81)	1.60 (1.33~2.19)
Dimethoate	560	120	2.54±0.24	3.12 (2.67~3.54)	9.95 (8.10~13.50)
			Aedes aegypti		
Imidacloprid	590	120	4.33±0.34	0.045 (0.042~0.048)	0.089 (0.079~0.10)
Tebufenozide	540	100	0.98±0.20	d	d
Aldicarb	590	120	13.6±0.14	0.27 (0.26~0.28)	0.33 (0.32~0.35)
Dimethoate	600	120	6.32±0.48	6.41 (6.02~6.85)	10.23 (9.23~11.89)

a : Column headings are abbreviated as follows : N is number of insects treated with a specific insecticide ; NC is number of test organisms treated with solvent only ; S. E. is standard error.

b : The equality tests between the slopes of two probit lines are performed as a routine of POLO-PC program. All four probit lines for the same species are different from each other ($p < 0.05$).

c : The LC values (mg/L) are given with the 95 % confidence interval in parentheses.

d : Less than 50 % mortality during 48 h test period

This study demonstrated that imidacloprid causes neurotoxic symptoms in mosquitoes similar to those reported earlier in many other insect species(7-8), and that it is more toxic than the two Ach esterase inhibitors. Mosquitoes in this study were more susceptible to imidacloprid than many species of Homoptera, Hemiptera, and Coleoptera(7).

In conclusion, there was a greater correlation of susceptibility to test chemicals within taxonomically close organisms than organisms that share habitats. *Aedes taeniorhynchus* was the most susceptible and *Artemia* sp. was the most tolerant species to insecticides tested. Both fresh water species were more tolerant to tebufenozide at 20 °C than at 27 °C. These results suggest that both tebufenozide and imidacloprid are selective insecticides with reasonable environmental safety toward non-target crustacean organisms.

2. Osmotic stress as an insecticide toxicity modifying factor on two euryhaline arthropods

The main objective of this study was to determine whether salinity contributes to the effect of these insecticides on two euryhaline arthropods. The tolerance of each organism to four insecticides was tested under iso-osmotic, and hyper-osmotic conditions. Materials and methods are the same as in the first study except iso-osmotic condition for each species.

Cumulative mortality after 48 h did not exceed 50% for either test species at iso-osmotic conditions, except for *A. taeniorhynchus* exposed to aldicarb (Fig. 3 and 4). Observations after 72 h provided LC50 data shown in Table 3. *Artemia* were more susceptible to the two Ach esterase inhibitors than to the two newer insecticides. Even after 72 h of exposure to imidacloprid or tebufenozide, mortality of *Artemia* nauplii at iso-osmotic condition did not exceed 50%. There was no significant difference in the tolerance of individual species to aldicarb and dimethoate, but *Artemia* was more tolerant (> 50x) to these chemicals than *A. taeniorhynchus*.

Table 3. The estimated 72-h LC50 values (mg/L) for test chemicals under iso-osmotic conditions.

Insecticides	*Aedes taeniorhynchus*	*Artemia* sp.
Imidacloprid	0.021 (0.017~0.030)	ND
Tebufenozide	0.35 (0.29~0.52)	ND
Aldicarb	0.20[a]	17.25[a]
Dimethoate	0.20[a]	10.14 (4.89~19.55)

a : The 95% confidence interval could not be determined due to large heterogeneity.
ND : Mortality was less than 50% over the concentration range tested in this study.

가. Comparison of iso- and hyper-osmotic conditions

Artemia tolerated higher concentrations (62.5 to 300 mg/L) of all four insecticides than *A. taeniorhynchus* (0.016 to 0.81 mg/L) (Fig. 5). Both species were more susceptible to all insecticides under hyper-osmotic conditions (Figs. 6 and 7). *Aedes*

taeniorhynchus generally had a greater variation in tolerance to test chemicals between iso- and hyper-osmotic conditions than *Artemia*. However, the two species had a different response to dimethoate, with mosquito larvae being more susceptible to this Ach esterase inhibitor at hyper-osmotic conditions than *Artemia*. This suggests that osmotic stress does not catagorically increase the toxicity of a chemical, but mortality is an animal and/or chemical specific response. There was an obvious time-dependent mortality in *Artemia* (Fig. 4), probably because *Artemia* is generally more tolerant to these insecticides than *A. taeniorhynchus*.

Hyper- and hypo-regulation of body fluids represent elaborate adaptations to salinity stress (9). Although the two species are able to live over a wide range of salinity, they are not insensitive to chemical pollutants in their environments. According to Croghan (10), the gut systems of both *Artemia* and *A. taeniorhynchus* have similar physiological function with relation to water balance. However, crustaceans do not possess an excretory system based on Malpighian tubules or rectal glands as do *A. taeniorhynchus*.

It is possible that there is decreased osmotic stress as external salinity conditions approach the iso-osmotic point of each organism. Even organisms highly adapted to wide salinity changes may be more susceptible to chemical exposure at hyper-osmotic than at iso-osmotic conditions. Therefore, it is reasonable to assume that higher salinity may require greater metabolic effort by organisms than at iso-osmotic conditions. Tebufenozide's action on the integument and imidacloprid's action on the nicotine receptors of these aquatic organisms might indirectly interfere with their ability to osmoregulate. Tebufenozide may initiate apolysis of the ectodermal hindgut epidermis in *A. taeniorhynchus* resulting in an impairment of ion elimination. Likewise, the action of imidacloprid on the caudal ganglia of the central nervous system would probably affect hindgut contraction and ion regulation.

In summary, *Artemia* were more tolerant to all chemicals tested compared to *A. taeniorhynchus* under iso-osmotic conditions; and simultaneous exposure to increased salinity and toxic chemicals elevated susceptibility in both species. In general the results indicate that *A. taeniorhynchus* is more susceptible to the tested chemicals than *Artemia* under the stress of hyper-osmotic conditions. This study has led us to hypothesize that euryhaline organisms might be more susceptible to chemicals in the field than LC50 data suggest, since other abiotic factors such as salinity, temperature, and oxygen availability may cause additional stresses.

3. The Influence of fluctuating salinity on insecticide tolerance of two euryhaline arthropods

Previous studies with static 48-h toxicity tests at hyper- and iso-osmotic salinities of both species (11) have shown that salinity was a modifying factor on insecticide induced mortality. However, salinity is continuously changing in nature and static toxicity tests may not represent estuary or similar salinity changing environments. Therefore, in this study, we developed a small flow-through system to expose test species to continuously changing salinity conditions in the presence of insecticides.

The flow-through system (Fig. 8) was designed to change salinity range from either direction: (1) hyper (200% ASW) to hypo (10% ASW), or (2) vice versa. In this system, a test solution with the appropriate salinity (either 10 or 200% ASW) and chemical concentration was pumped from its source to mixing jar 1, then pumped into mixing jar 2, and exposure containers(total volume = 50 mL) consecutively. The entire closed system was regulated by a Masterflex® pump (Cole-Parmer Instrument Co., IL) with two channels which maintained a flow rate of 1.43 mL/min.

Salinity was estimated based upon the measured turnover time of exposure containers and compared with the actual salinity change measured by a vapor pressure osmometer (Wescor Inc., Logan, Utah). ASW contains Na^+(0.45M), K^+ (0.01M), Mg^+ (0.052M), and Cl^-(0.53M). The concentration of each chemical used was based on LC50 in 48 h static toxicity tests in hyper-osmotic conditions (200% ASW) for both species (Table 1). Four groups of 15 larvae or nauplii were acclimated in the medium of either 10% or 200% ASW at room temperature for 1 h prior to testing. Then they exposed to ASW containing an appropriate concentration of toxin.

Data are presented in the text and shown in figures as mean standard error (SE = error bars in figures). Statistical comparisons between experimental data were made with Students t-test. Cumulative mortality curves at either salinity change were compared statistically using orthogonal polynomials in the procedure of repeated measures analysis of variance (ANOVA).

The simulated values agreed closely with actual concentration changes measured by an osmometer ($p < 0.05$) (Fig. 9). Cumulative mortality of both species was greater as salinity increased (10 to 200% ASW) compared to decreasing salinity environments (200 to 10% ASW) except tebufenozide to *A. taeniorhynchus* (Figs. 10-11). However, the mortality rate based on LT50 was more acute for *A. taeniorhynchus* in decreasing

salinity than in increasing salinity, but it was more acute for *Artemia* in opposite direction of salinity change(Fig. 11). In decreasing salinity, *A. taeniorhynchus* was more susceptible than *Artemia* except dimethoate to *A. taeniorhynchus*, since *Artemia* showed near 50 % mortality for 48 h. In increasing salinity, both species was more susceptible to all tested chemicals compared to the result at hyper-osmotic conditions (Table 1), since they showed 50% mortality before 48 h. Generally, *A. taeniorhynchus* always reached or approached 50% mortality sooner than *Artemia* sp., regardless of the direction of the change in salinity experienced. With the exception of dimethoate (Fig. 10a), *A. taeniorhynchus* was more susceptible to insecticides in conditions of decreasing salinity. LT50 was decreased from 48 h at static hyper-osmotic salinity (11) to 18-30 h as the result of experiencing a salinity change concurrent to exposure to chemicals. These results indicate that fluctuating salinity, especially increasing salinity, acts synergistically with insecticides to cause greater toxicity to mosquito larvae.

The mortality in *Artemia* populations exposed to conditions of decreasing salinity (200 to 10% ASW) over 48 h was identical to earlier estimated LC50 values in a static hyper-osmotic condition (Table 1). However, mortality increased dramatically, exceeding 80% in test with all three chemicals when the change in salinity was in the opposite direction (10 to 200%). The time until all tested chemicals killed half the population (LT50) was also hastened by increasing salinity conditions.

These results suggest that mortality to a given concentration of an insecticide is higher under conditions of changing, salinity specially increasing salinity, than previously reported in static bioassays. Several explanations appear plausible for the elevated mortality observed in the current study. As described previously, both organisms were reared in 100% ASW then acclimated to salinities (either 10% or 200% ASW) of test media for 1 h prior to the toxicity test. This means that the greatest change in hemolymph osmotic pressure may coincide with the initial exposure to an insecticide, thereby aggravating the stress to the animal. The results provide evidence that sudden changes in salinity can lower the limit of insecticide tolerance of these test species.

Why were these organisms more susceptible to increasing than to decreasing salinity? Both larvae and adults of brine shrimp are excellent hypo-regulator at higher salt concentrations. In concentrated brine, *Artemia* still maintains its body fluid at an osmotic pressure at nearly one-tenth that of the medium, but it cannot survive in fresh water (12-13). This may be

the reason why *Artemia* was more tolerant to insecticides in decreasing than in increasing salinity; since it is a better osmoregulator in more concentrated medium. On the other hand, although *A. taeniorhynchus* develop normally in all salt concentrations from distilled water to 150% sea water, their growth can be retarded in salinities between 150% and 300% (14). Earlier studies (14-15) have shown that *A. taeniorhynchus* grow optimally at salt concentrations toward the lower end of this wide tolerance range. It is not surprising that mosquito larvae were less tolerant to insecticides in increasing salinity conditions (100 to 200% ASW), since *A. taeniorhynchus* is a better osmoregulator at lower salinity (150% sea water). Therefore, for each chemical, with the exception of dimethoate (Fig. 10a), the LT50 of *A. taeniorhynchus* was shorter in populations exposed to decreasing salinity as compared to increasing salinity. Due to extreme susceptibility of *A. taeniorhynchus* to these insecticides, there were smaller changes in its cumulative mortality compared to *Artemia*, regardless of the direction of salinity changes.

Considering salinity change as one of the unique abiotic characteristics of saline habitats, the flow-through system in this study was logical since it uses conditions more reflective of natural habitats. This system can be an alternative way of assessing the potential toxicity of a chemical to a saline ecosystem without endangering the system itself. It is also inexpensive, requires minimum space, and is easily manipulated. A manipulation of any one and/or more components in this system would give different salinity fluctuation patterns (Fig. 12), reflecting osmotic stress imposed on animals under various salinity conditions.

This study exhibits that a species, which in the laboratory can easily withstand a wide range of salinity, may not survive small changes in the field if these changes are associated with other stress factors. This study demonstrates that one such factor is the presence of pollutants in the form of insecticides. This study clearly confirms that salinity change and its direction of change aggravate the stress on an organism already experiencing exposure to toxic insecticides, so lowering the limit of insecticide tolerance of test species. This flow-through system can be used prior to a field trial to approximate the chemicals toxicity based on the expected changes in salinity characteristic of each estuarine ecosystem targeted for pesticide exposure.

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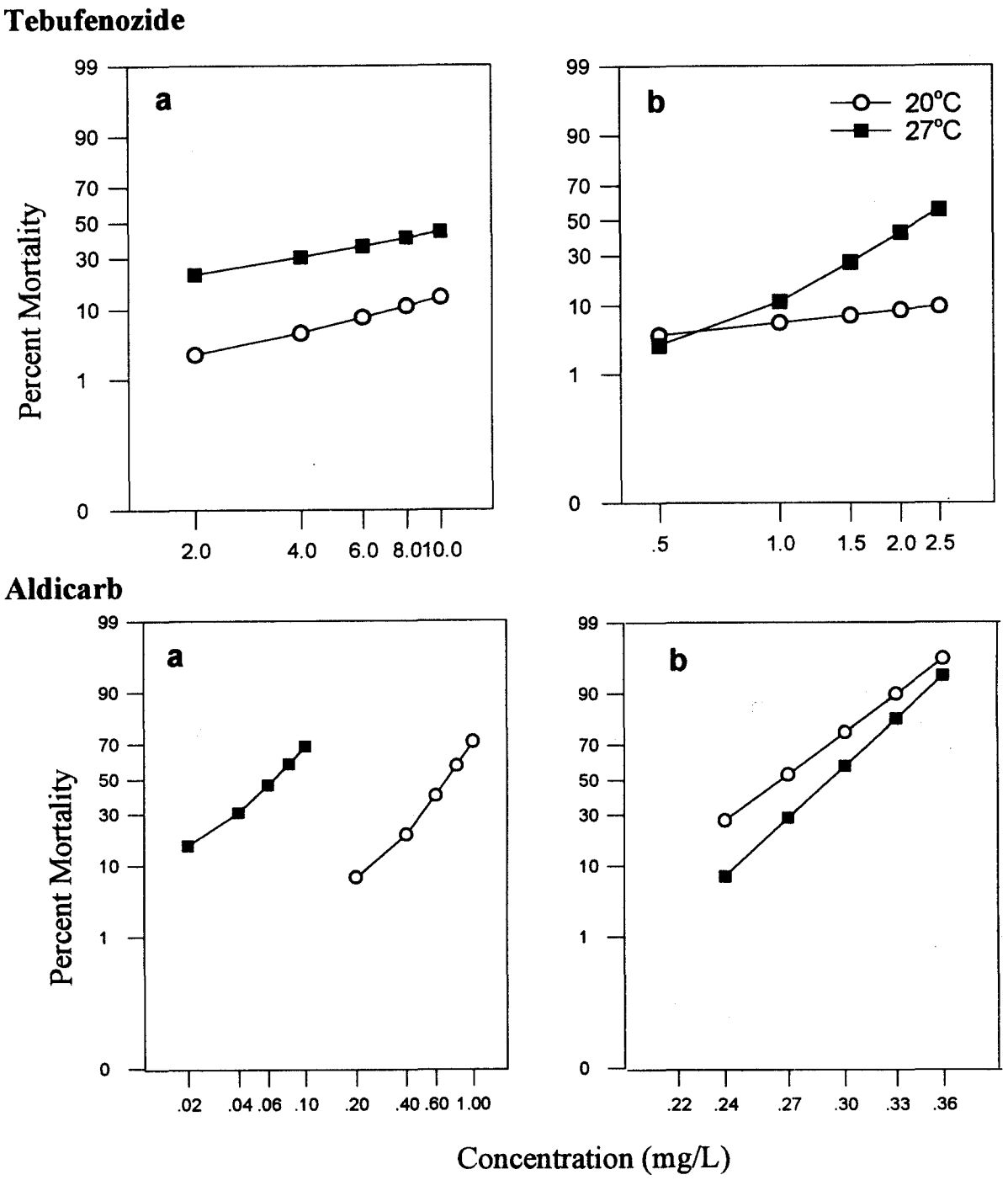

Fig. 1. Percent mortality when *Daphnia magna* (a) and *Aedes aegypti* (b) were exposed to tebufenozide and aldicarb at two temperatures for 48 h. Percent mortality and concentration were plotted as probit versus concentration on a logarithmic scale.

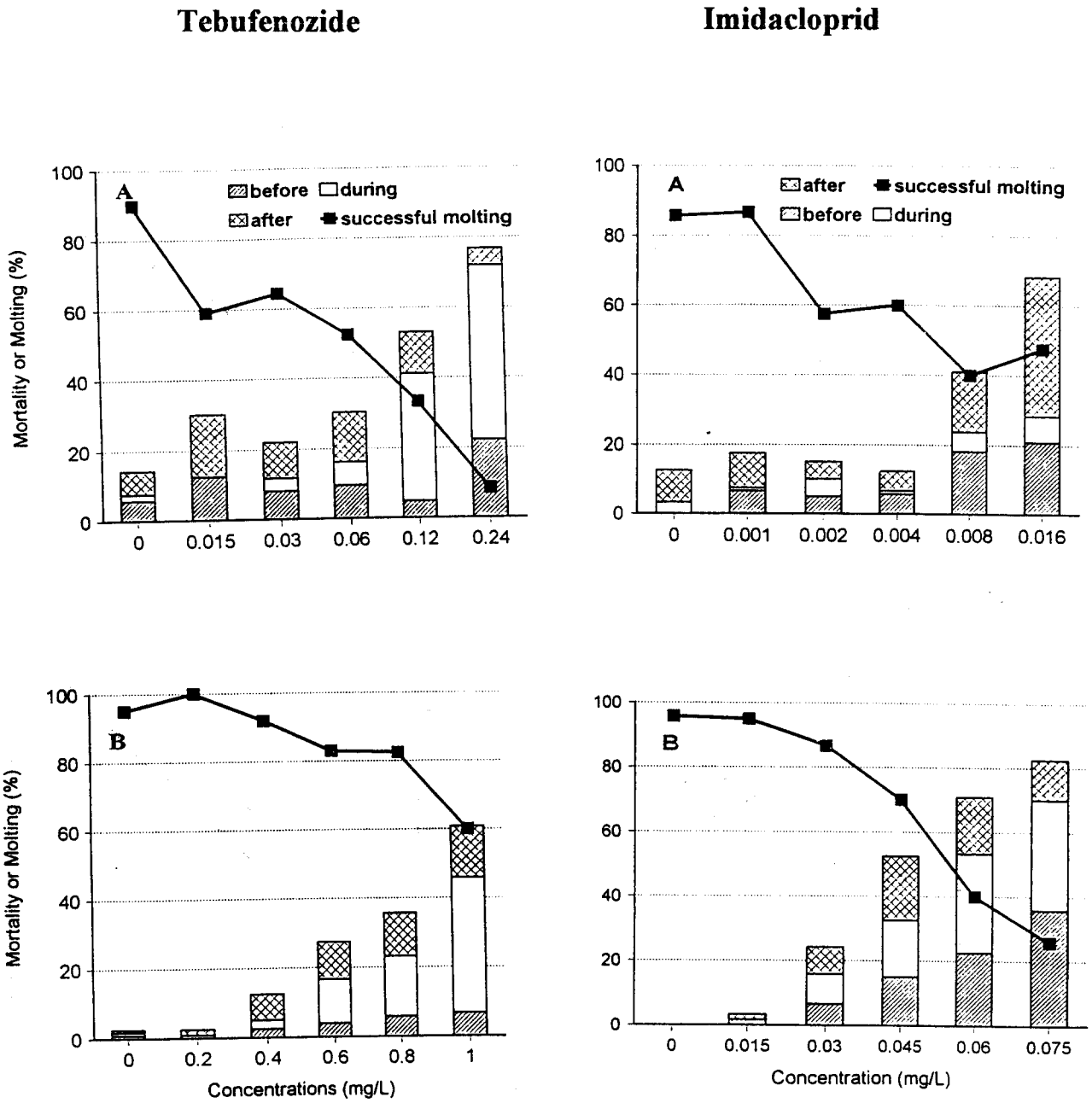

Fig. 2. The occurrence of molt-related mortality in mosquito larvae 48 h after exposure to tebufenozide and imidacloprid: *Aedes taeniorhynchus* (A) and *Aedes aegypti* (B).

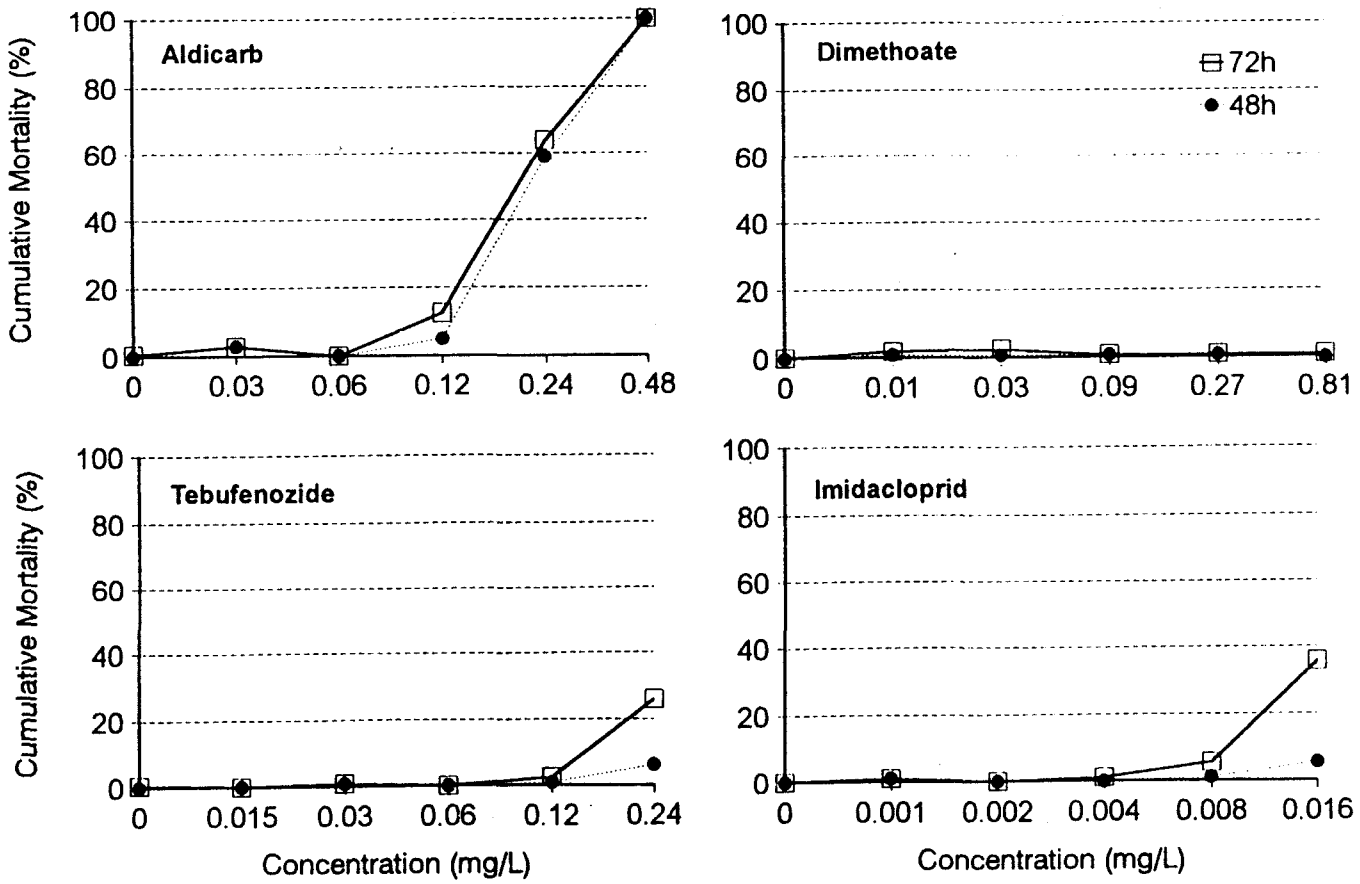

Fig. 3. Cumulative mortality of *Aedes taeniorhynchus* exposed to a range of insecticide concentrations under iso-osmotic conditions

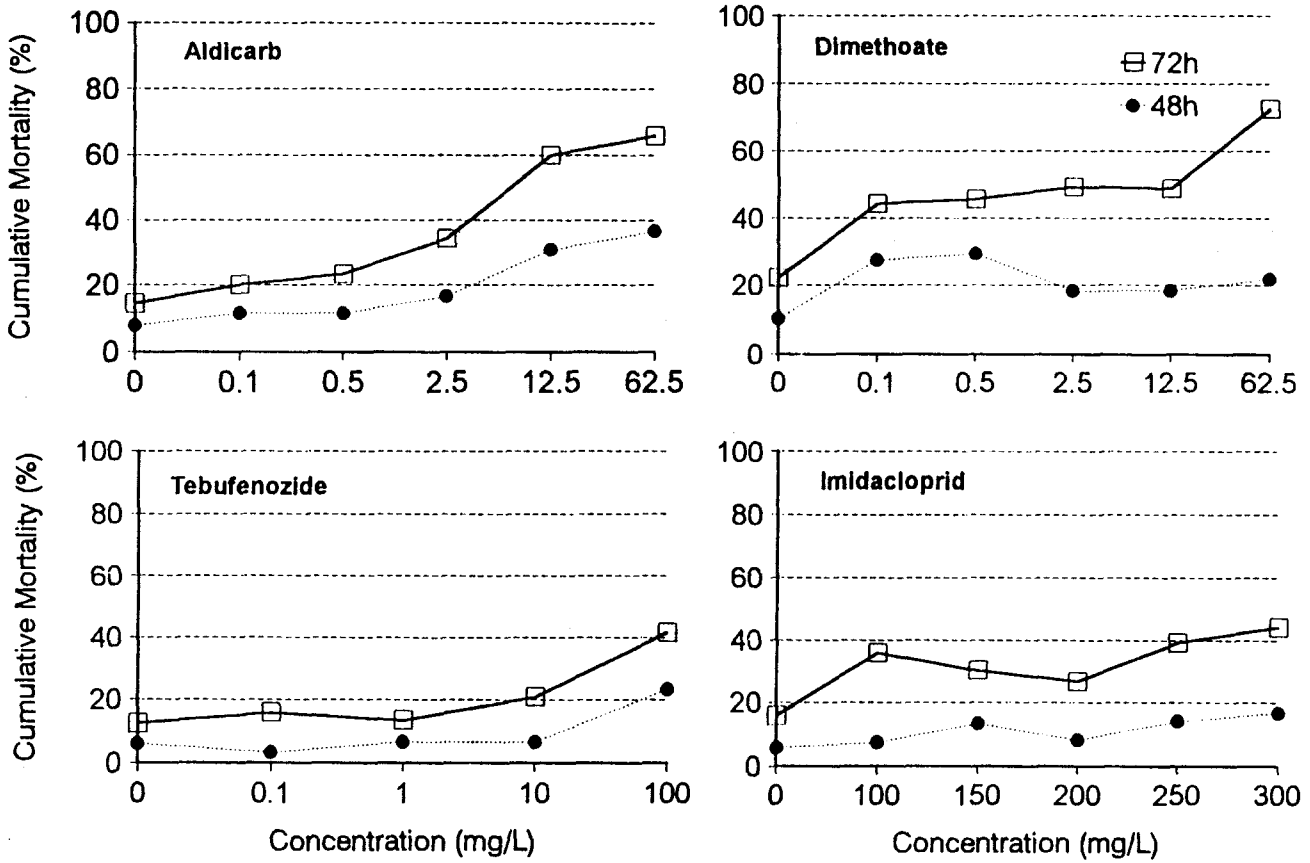

Fig. 4. Cumulative mortality of *Artemia* sp. exposed to a range of insecticide concentrations under iso-osmotic conditions

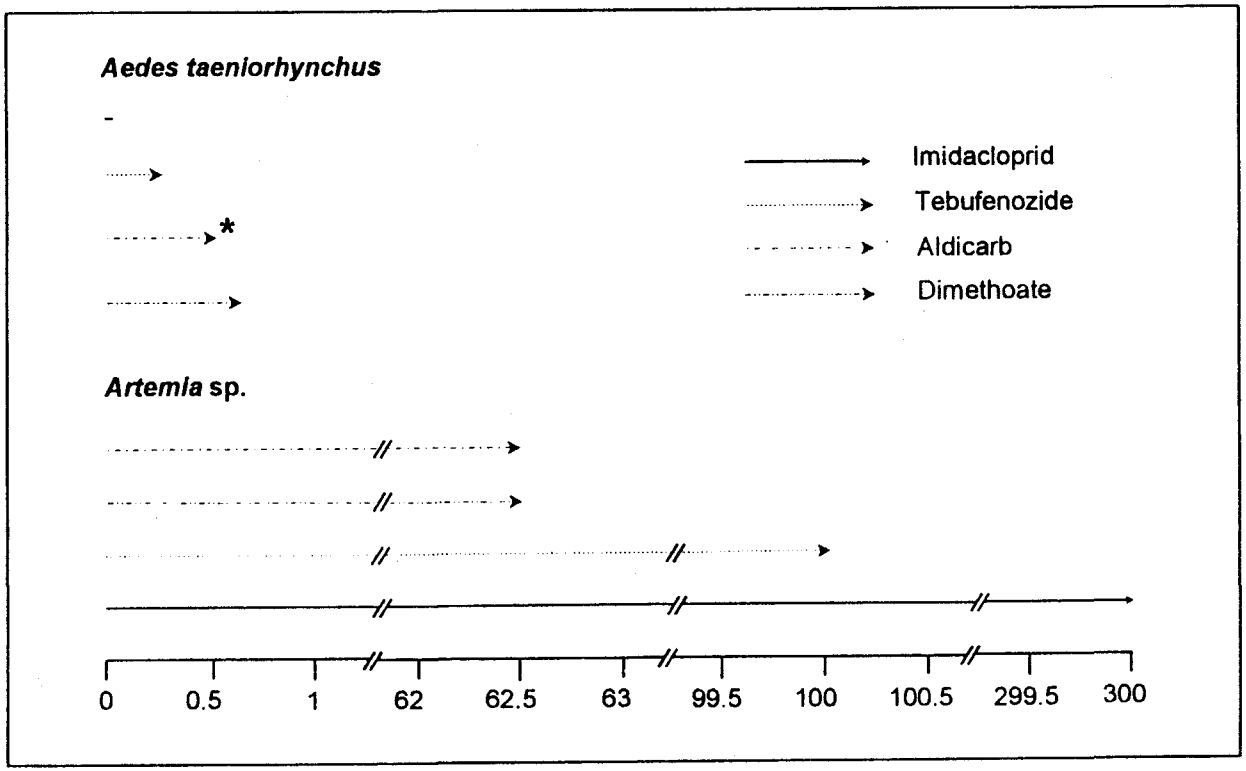

Fig. 5. Experimental design for the concentration ranges (mg/L) of chemicals tested for two euryhaline species at both iso- and hyper-osmotic conditions. The asterisk denotes the only chemical (Aldicarb) that caused more than 50% mortality at iso-osmotic condition during 48-h toxicity tests.

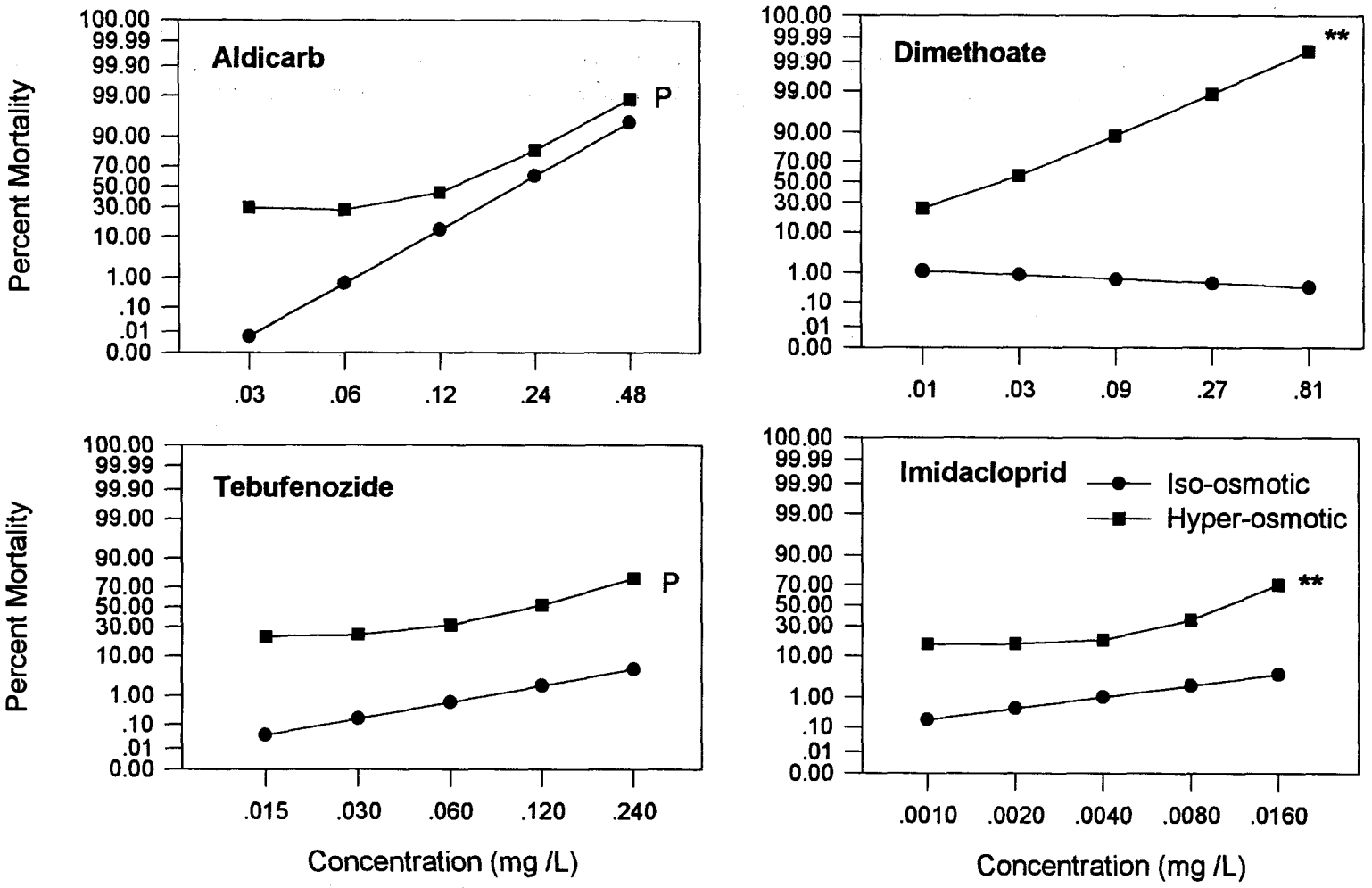

Fig. 6. Mortality of *Aedes taeniorhynchus* exposed to various concentrations of four insecticides at two levels of salinity for 48 h. P means that slopes of two lines are the same. Asterisks mean that slopes of two lines for the same chemical tested at two different levels of salinity are significantly different (** $P < 0.01$). Mortality and concentration were plotted as probit versus concentration on a logarithmic scale.

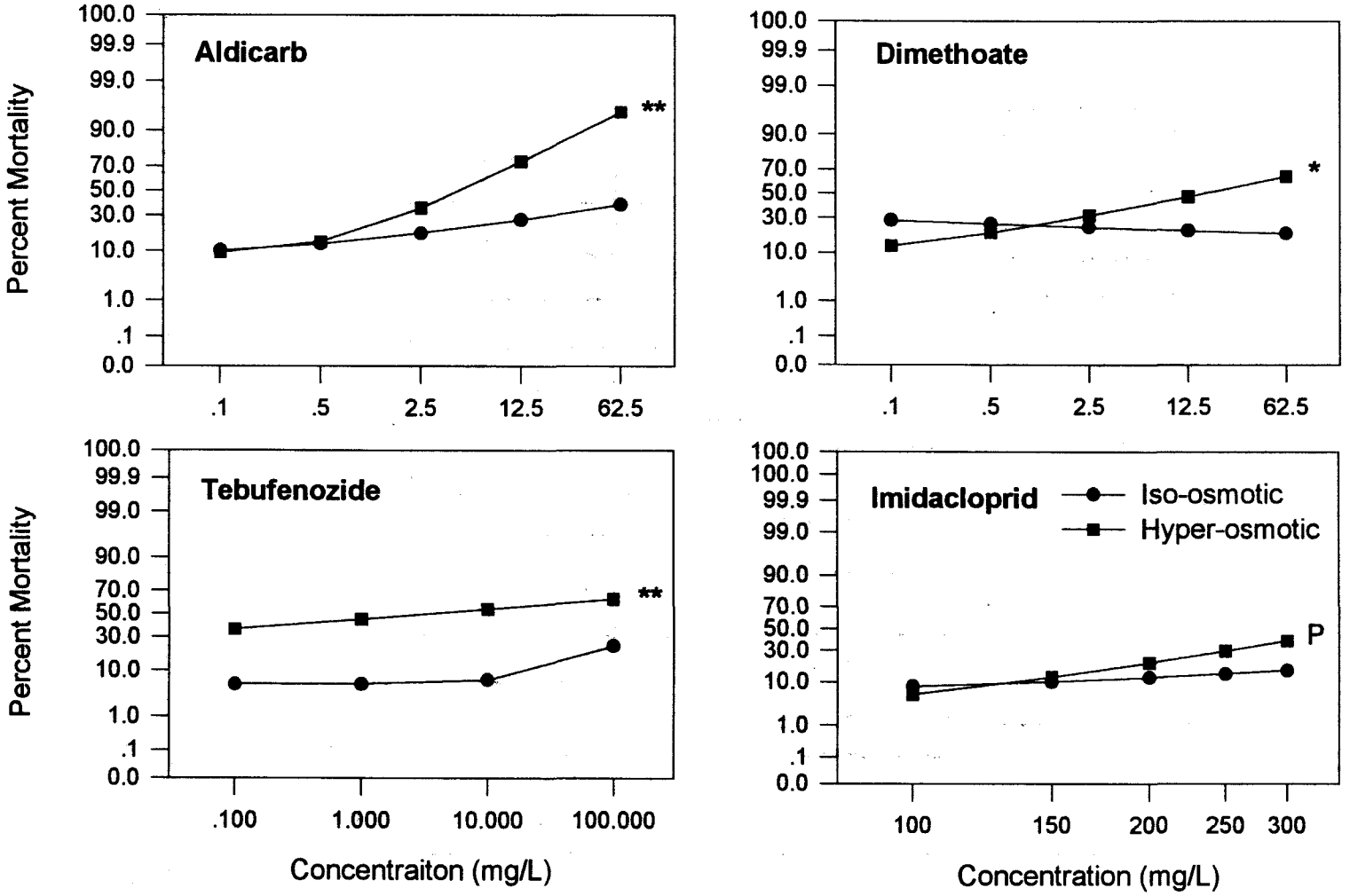

Fig. 7. Mortality of *Artemia* sp. exposed to various concentrations of four insecticides at two levels of salinity for 48 h. P meansthat slopes of two lines are the same. Asterisks mean that slopes of two lines for the same chemical tested at two different levels of salinity are significantly different (*, $p<0.05$;** $P < 0.01$). Mortality and concentration were plotted as probit versus concentration on a logarithmic scale.

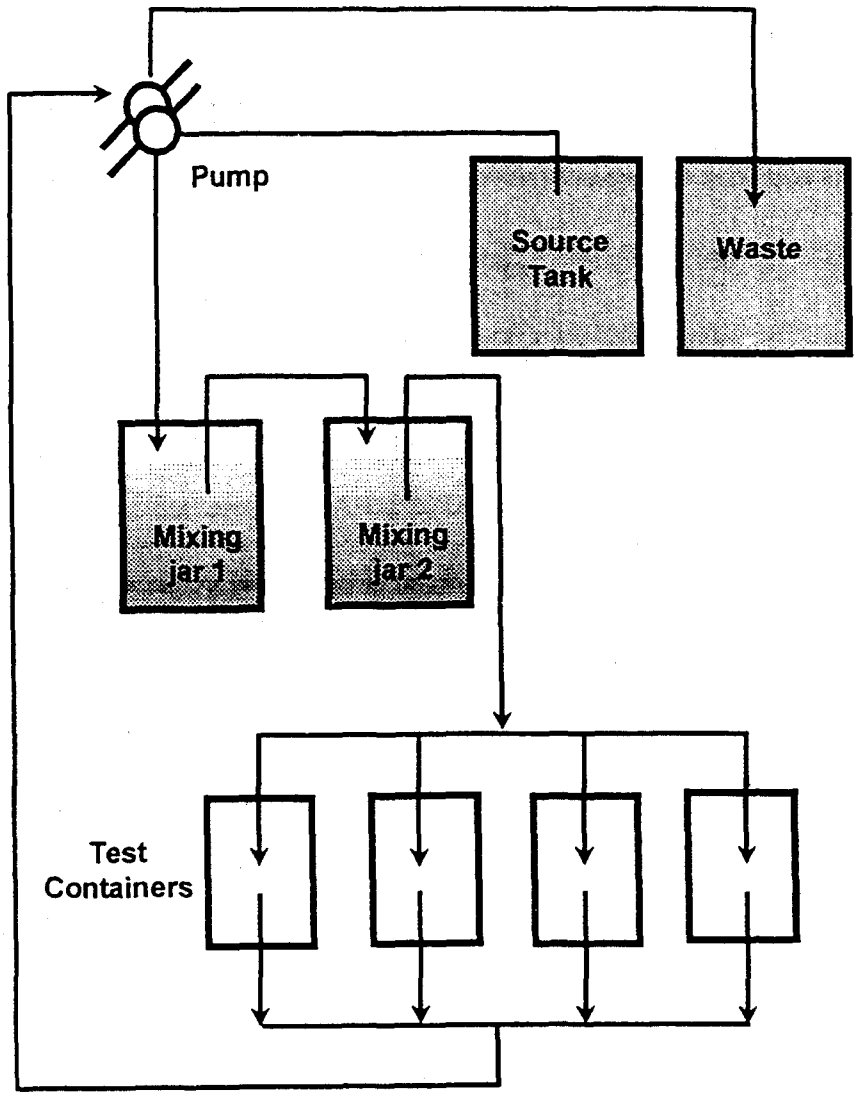

Fig. 8. A simplified diagram of a flow-through system that changes the salinity. Exposure containers are four replicates of the same toxicity test.

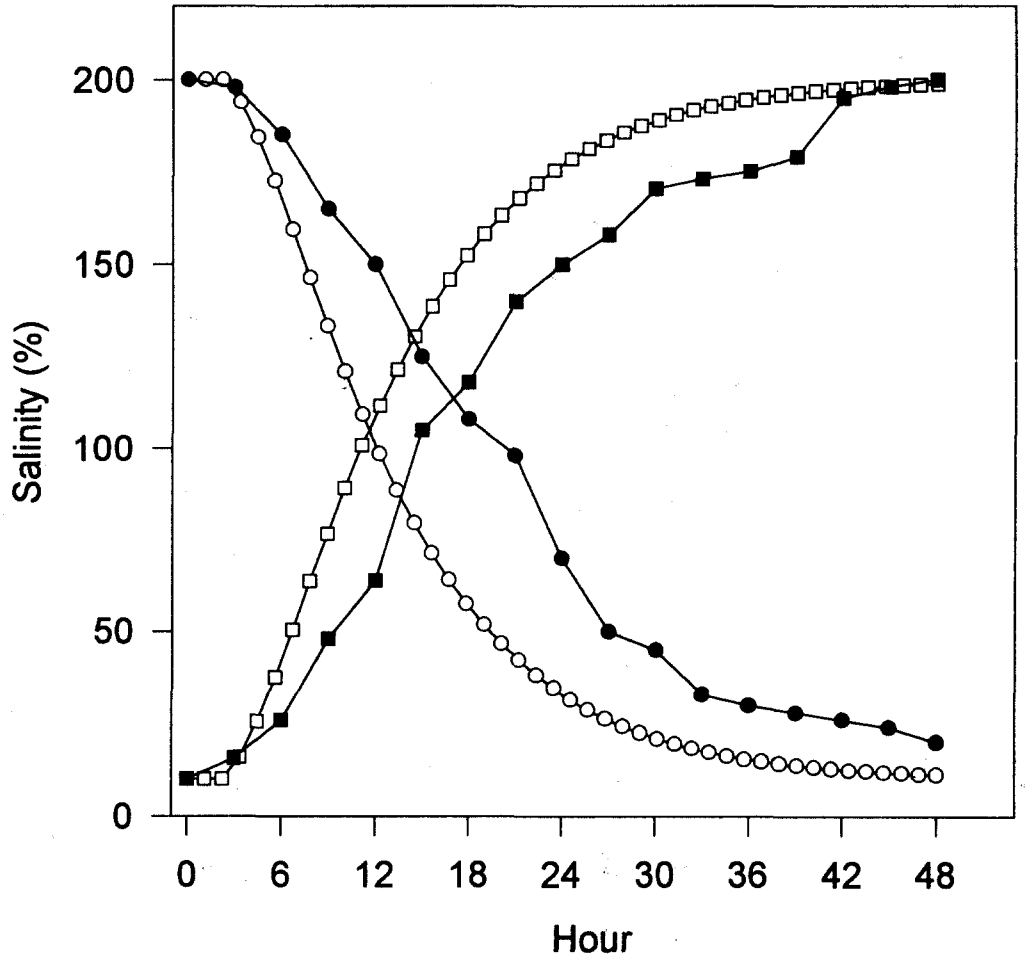

Fig. 9. The simulated salinity changes (open) compared to the measured concentrations (closed). All data in measured concentrations represent mean values of each test solution (n=10). The differences between two curves in each direction were not found to be statistically significant ($p > 0.05$).

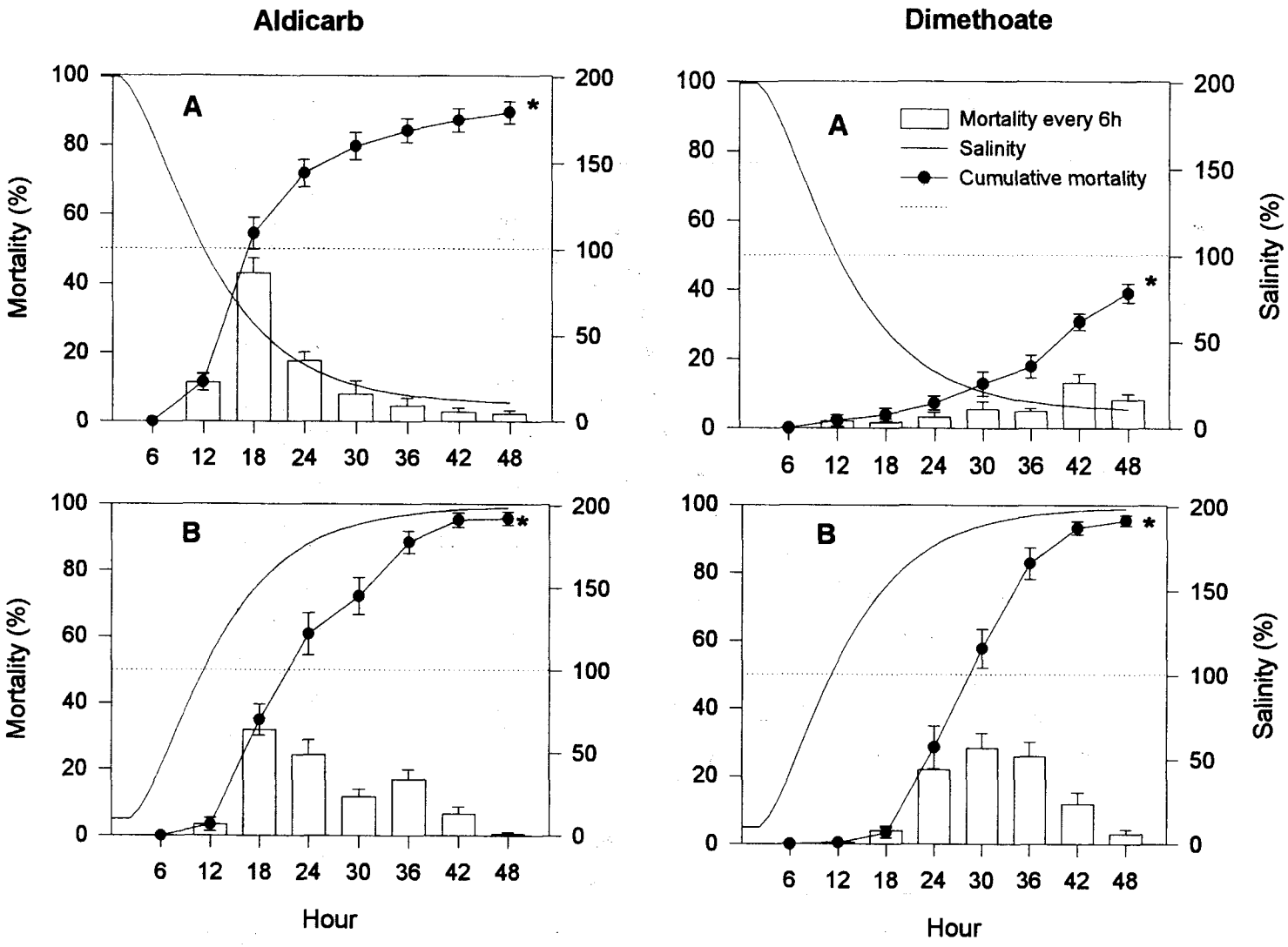

Fig. 10a. Comparison of *Aedes taeniorhynchus* mortality after exposure to aldicarb (0.15 mg/L) and dimethoate (0.031 mg/L) under changing conditions of salinity. Salinity changes tested were from 200 to 10% (A) and from 10 to 200% sea water (B). Asterisk denotes a significant difference between the mortality curves in A and B (*, $p < 0.05$).

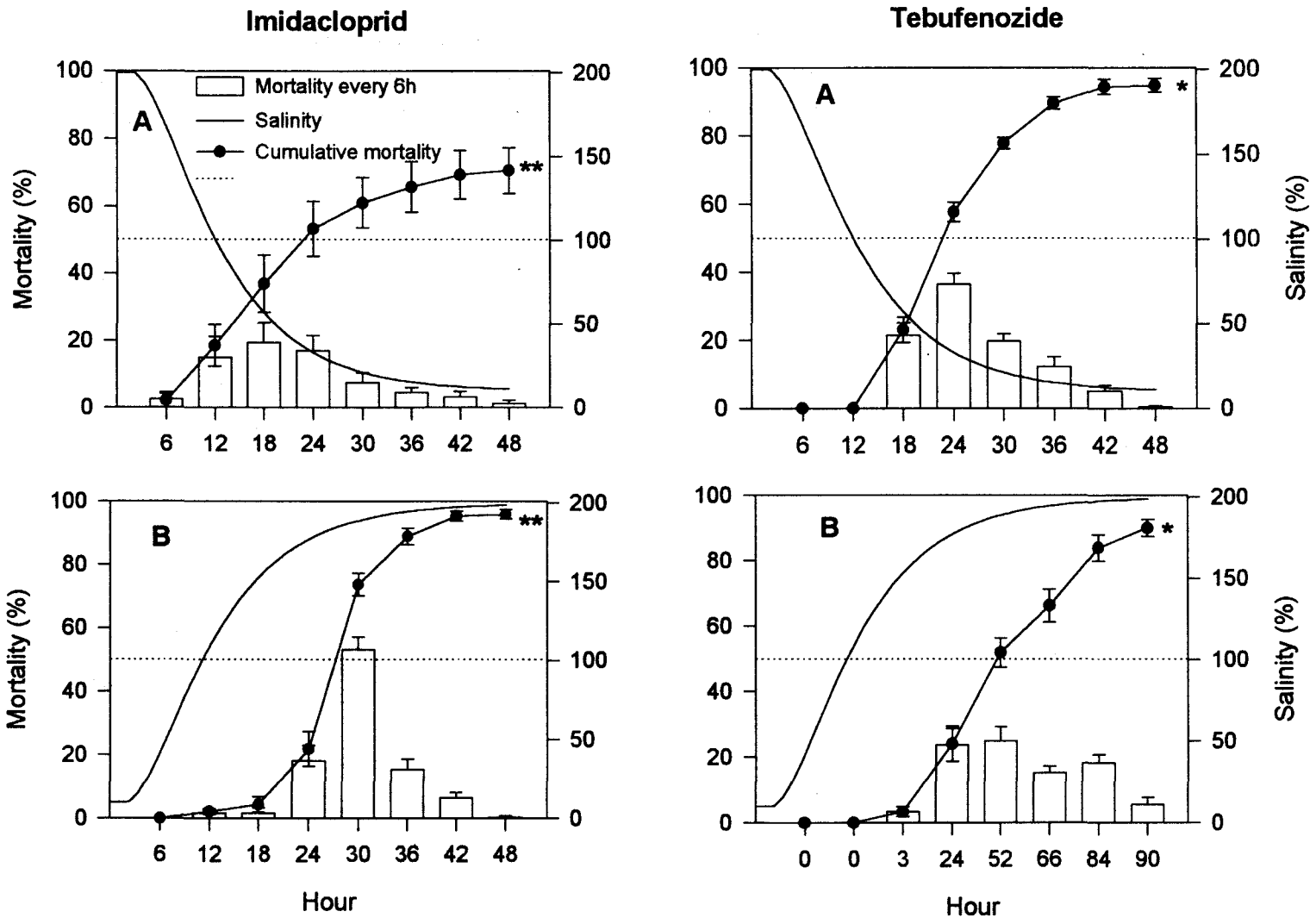

Fig. 10b. Comparison of *Aedes taeniorhynchus* mortality after exposure to imidacloprid (0.013 mg/L) and tebufenozide (0.15 mg/L) under changing conditions of salinity. Salinity changes tested were from 200 to 10% (A) and from 10 to 200% sea water (B). Asterisk denotes a significant difference between the mortality curves in A and B (*, $p < 0.05$;**,$p<0.01$).

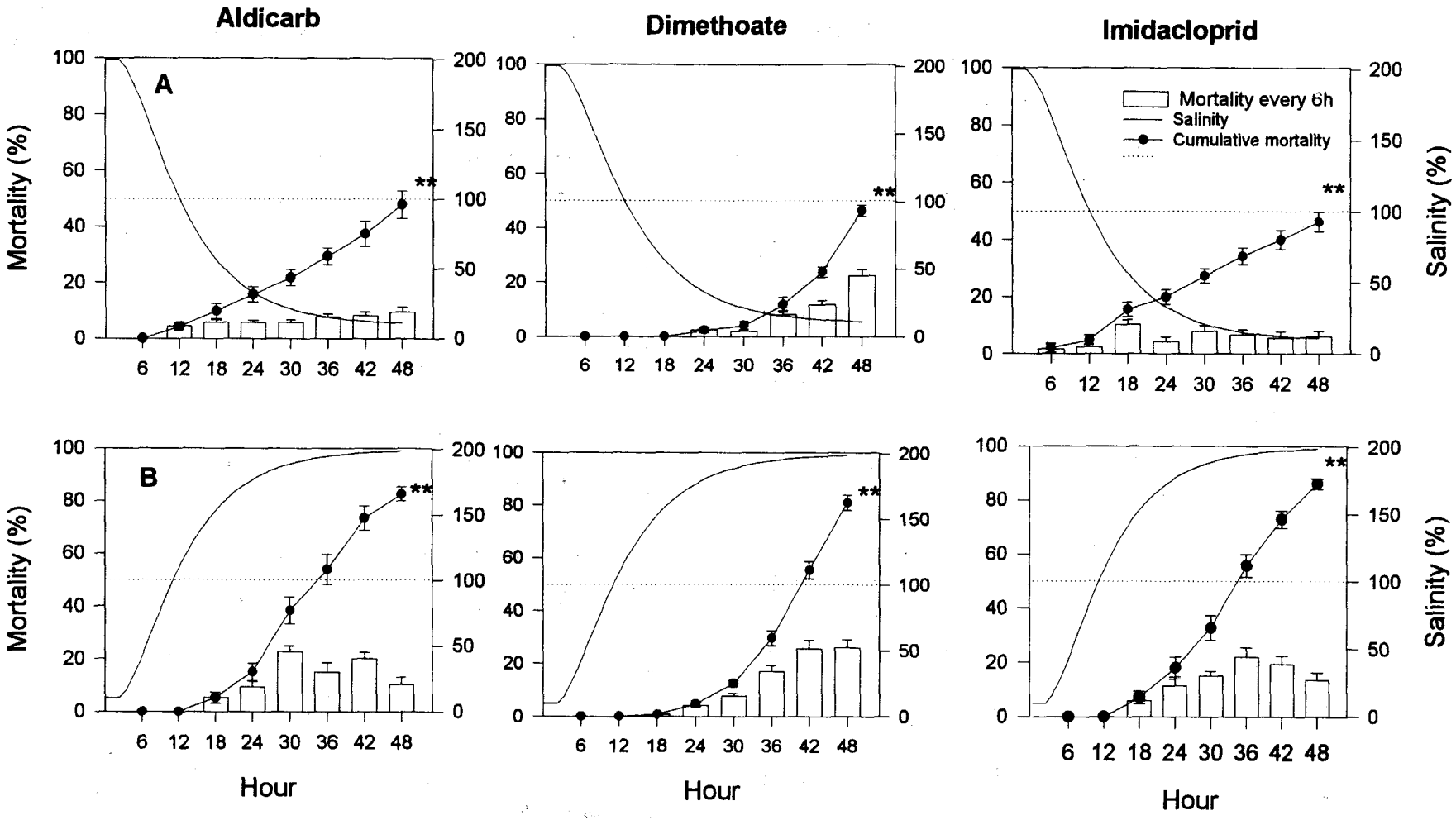

Fig. 11. Comparison of *Artemia* mortality after exposure to aldicarb (5.46 mg/L), dimethoate(15.4 mg/L) and imidacloprid (361.23 mg/L) under changing conditions of salinity. Salinity changes tested were from 200 to 10% (A) and from 10 to 200% sea water (B). Asterisk denotes a significant difference between the mortality curves in A and B (**, $p < 0.01$).

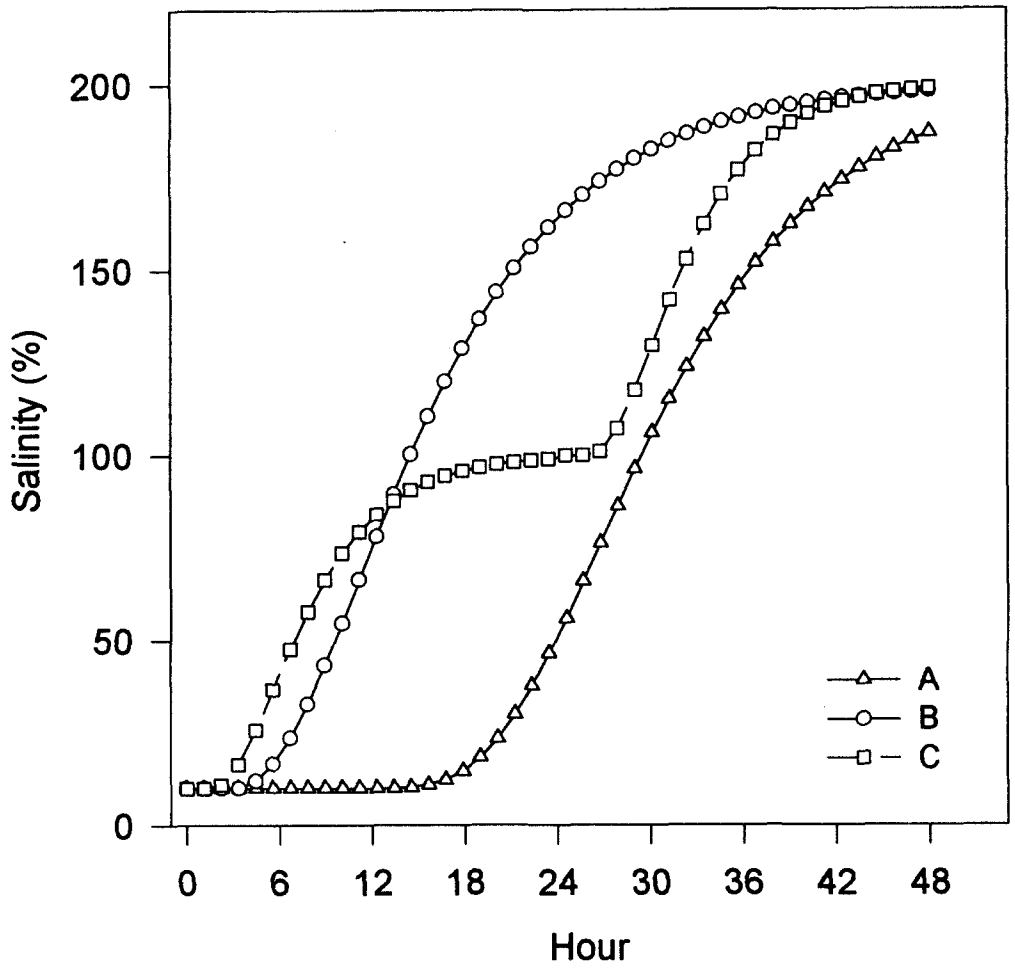

Fig. 12. Examples of increasing salinity changes by modifying some components in the flow-through system. A: 10 mixing jars of 10% ASW; B: 3 mixing jars of 10 % ASW; C: the same condition as in the case of B, but using twice the flow rate than B and switching source tank from 100% to 200% ASW after 24 h.