The Influence of Cations on Chlorine Toxicity

Glenn M. Cohen
Department of Biological Sciences
Florida Institute of Technology
Melbourne, Fla. 32901

Fish are known to be extremely sensitive to chlorine, though the underlying basis of this sensitivity is incompletely understood (BRUNGS 1973). A probable explanation for piscine sensitivity to chlorine is attributable to impairment of gill function, since other pollutants such as copper sulfate (BAKER 1969), and sodium dodecylbenzenesulfonate (SCHMID and MANN 1961) directly damage gill epithelium and underlying cells. PENZES (1972) reported that chlorine caused separation of epithelial layers within the gill. In addition, VALENZUELA (1976) showed that in mosquito fish once chlorine disrupted the gill's permeability barriers the severity of damage to underlying cells was in large part related to ionic and osmotic stresses of water, though chlorine also directly damaged exposed cells. Thus chlorine caused minimal gill damage and lowest mortalities in 25% sea water, which was closest ionically to body fluids of the waters tested (PROSSER 1973). Accordingly in the present experiments, the moderately euryhaline mosquito fish were exposed to chlorine in waters of different ionic compositions and concentrations to determine whether intoxication is the physiological counterpart to the gill's structural damage (VALENZUELA 1976) and, conversely, whether recovery from intoxication, even if temporary, is possible in ionically balanced media. In short, there were marked differences to both intoxication and recovery among these waters.

MATERIALS AND METHODS

Mosquito fish, Gambusia affinis, served as the test animals, as described elsewhere (KATZ and COHEN 1976). The mosquito fishes' sensitivity to chlorine was compared in test waters composed of different ionic compositions and concentrations, which are listed in Table I. Deionized water was used for dilutions of sea water (S.W.) and for preparation of all test waters to which cationic salts were added. Tap water (T.W.) was dechlorinated by exposure to sunlight prior to use. Chlorine was prepared by diluting commercial bleach (Clorox ®, 5.25% NaOCl).

Using procedures as outlined in Standard Methods (AMERICAN PUBLIC HEALTH ASSOCIATION), levels of free and combined residual chlorine were determined by the orthotolidine and sodium arsenite methods; hardness, alkalinity and chlorides were measured for water chemistry analyses. Sodium and potassium concentrations were determined on a Jarrell-Ash Atomsorb (Model 82-270) atomic absorption spectrophotometer in the emission mode.

Bulletin of Environmental Contamination & Toxicology,
Vol. 18, No. 2 © 1977 by Springer-Verlag New York Inc.

For these studies the static bioassay was used instead of continuous flow because it allows concurrent toxicity and dissipation measurements. Unless otherwise stated, mortalities were carried out for six days. Controls accompanied all toxicity measurements. Whenever the recuperative potential of a test water was to be determined, the fish remained in the original water at least one day before they were transferred. All experiments were conducted at room temperature (21±2°C). The fish were not fed during the course of experiments.

TABLE I

CATIONIC COMPOSITIONS OF TEST WATERS IN PPM

Test Water	Na+	Mg++	Ca++	K+
tap	70	14	64	5
0.125% NaCl	495	---	---	---
0.9% NaCl in tap	3,600	14	64	5
isotonic saline	3,400	144	160	234
10% sea water	1,000	130	40	38
25% sea water	2,500	325	100	95
50% sea water	5,000	650	200	190
100% sea water	10,000	1,300	400	380

RESULTS

The toxicity of chlorine to the mosquito fish Gambusia affinis, which are moderately euryhaline, is compared in waters of different cationic compositions and concentrations (TABLE I): T.W., dilute and normal S.W., and dilute sodium chloride. As shown in Fig. 1, toxicity to 0.5 ppm chlorine was lowest in 25% S.W. and maximal in T.W. and in 50% S.W., the latter two representing respectively the lowest and highest ends of the osmotic gradients. Although raising the chlorine dosage to 1.0 ppm increased toxicities in all waters, the fish in 10% and 25% S.W. showed the lowest toxicities; in the other waters the fish continued to show the highest toxicities (Fig. 1).

Placing the fish in a saline solution (TABLE I) believed to be the ionic equivalent of the blood (PROSSER 1973) proved to be highly stressful as indicated by mortalities (Fig. 2). Surprisingly, the dosing of chlorine into this physiological saline actually reduced mortalities rather than raised them (Fig. 2). It was subsequently deduced that potassium was largely responsible for these increased mortalities (Figs. 2 and 3). The experiments to test potassium toxicity more directly involved exposure to waters that were enriched with potassium or sodium alone (2 mM), or both together in equimolar concentrations (2 mM). The same sequence was followed for chlorine dosings (0.5 ppm) to these waters. It was demonstrated (Fig. 3A' and B') that chlorine reduced potassium toxicity at the concentration used.

In order to determine whether a water which lowered mortalities also confers any residual protection, mosquito fish, were first acclimated in 25% S.W. before being transferred directly to chlorinated T.W. (0.5 ppm); no mortalities occurred after the transfer (Fig. 4). But when fish acclimated to T.W. were then transferred to chlorinated T.W. (0.5 ppm), 50% died (cf Fig. 1).

In order to determine whether sodium specifically lessened or counteracted chlorine toxicity, 9 gm/l NaCl was mixed in T.W. and then dosed (—>) at 0, 1, and 24 hrs with 2 ppm chlorine (Fig. 5). Fig. 5A illustrates levels of total chlorine residuals during the experiments. It was found that sodium protected against chlorine toxicity (Fig. 5B). There were no fish deaths during the first 24 hrs in these heavily chlorinated waters. However, 3 fish died by 48 hrs and 11 more by 72 hrs. By comparison, all fish died in less than 8 hrs in similarly chlorinated T.W.

Since chlorine toxicity varied markedly in different waters, the question remained whether acutely intoxicated fish could recuperate in waters of appropriate ionic composition. Fish were left in T.W. that contained 1 ppm residual chlorine until "disequilibrated" (DANDY 1972) and then placed in T.W., 25%, 50%, or 100% S.Ws. TABLE II shows that after 2 hrs the highest percentage of fish recovered from disequilibrium in 25% and 50% S.Ws.; 1/7 recovered in T.W. and all the fish died in 100% S.W. By comparison, those intoxicated fish that remained in 100% S.W. for shorter periods (15-60 mins) before being transferred to T.W. displayed improved recoveries (TABLE II). When survivors remained overnight in their respective recuperative waters, those in 25% S.W. showed the lowest mortalities. The surviving fish actively swam and showed no further symptoms of disequilibrium.

TABLE II

RECOVERY OF INTOXICATED FISH IN DIFFERENT WATER

Composition of Intoxicating Water	Composition of Recuperative Water	Success of Recovery	
		2 hrs.	Overnight
1 ppm Chlorine in Tap	tap	1/7	1/7
	25% S.W.	6/7	4/7
	50% S.W.	6/7	0
	100% S.W.	0	-
	100% S.W. (15-60 mins) then transferred to tap.	7/7	3/7

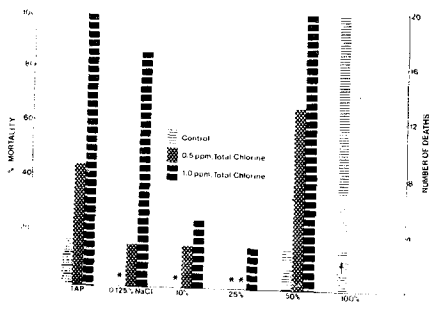

Fig. 1

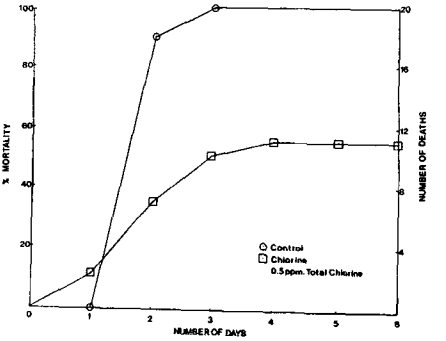

Fig. 2

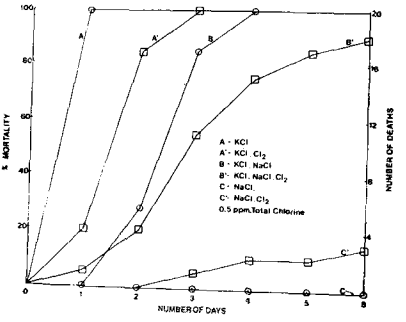

Fig. 3

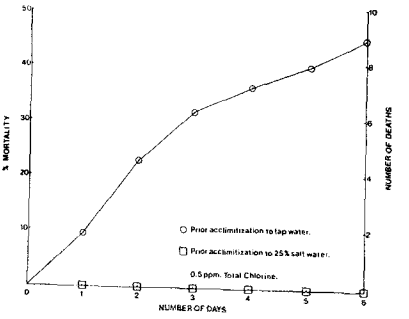

Fig. 4

Fig. 1. Fish mortalities in waters of varying ionic strengths and compositions during a six day period before and after exposure to chlorine. *No deaths during experimental period. †100% mortality in less than one day.

Fig. 2. Fish mortalities in piscine physiological saline before and after exposure to 0.5 ppm chlorine. Toxicity was lowered after exposure to chlorine.

Fig. 3. Fish mortalities in potassium and potassium-sodium enriched waters before and after exposure to 0.5 ppm chlorine. Toxicities were lowered after exposure to chlorine in A', B'.

Fig. 4. Differences in mortalities to 0.5 ppm chlorine in tap water between those fish first acclimated to 25% S.W. prior to transfer to chlorinated tap and those fish only acclimated to tap prior to transfer to chlorinated tap.

DISCUSSION

PENZES (1972) first reported that chlorine damages the gill epithelium, and possibly the underlying cells. More recently, VALENZUELA (1976) demonstrated that chlorine not only damages the

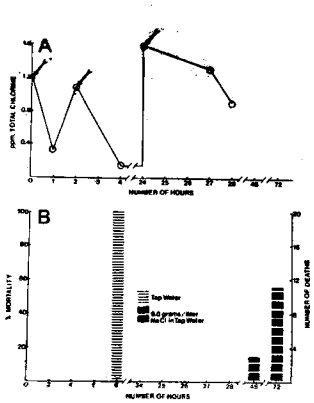

Fig.5

Fig. 5. Relation between chlorine dissipation and fish mortality in sodium-enriched (0.9%) tap.
A. Chlorine concentrations. Arrows (→) indicate time of chlorine dosings.
B. Comparison of mortalities and times of mortalities in normal and sodium-enriched tap waters. Initial mortalities were markedly reduced in the sodium-enriched water.

cells of gills but also causes the separation of the highly interdigitated surface epithelial cells, thereby exposing the underlying cells directly to the ionic and osmotic stresses of the external medium. Accordingly, after the exposure to chlorine, the gills swelled in hypotonic T.W. and shrank in the hypertonic (50% and 75%) S.W.; in the approximately isotonic 25% S.W. the damaged gills largely retained their normal integrity.

The present results, the physiological counterpart of the fine structural studies, demonstrate that cations markedly modify chlorine toxicity to the mosquito fish Gambusia affinis. Whereas the first set of experiments illustrates the relative toxicities of chlorine in different media (Figs. 1-5), the second set compares the success of initial recoveries from intoxications (Table II). In Fig. 1 it can be seen that 25% S.W. and 0.125% NaCl most effectively reduce toxicity to 0.5 ppm chlorine over controls, in comparison to tap or other waters. When chlorine concentrations were raised, mortalities increased in all waters, but to a lesser extent in 10% and 25% S.W. Fish previously acclimated to the 25% S.W. before transfer to T.W. were significantly more resistant to chlorine than fish acclimated to T.W. (Fig. 4). This increased resistance is intriguing because the cationic levels in T.W. should have been too low to have protected the fish. Interestingly, the mortality is almost identical to fish maintained in chlorinated 25% S.W. The basis of this residual or latent protection is conjectural presently.

The greatest increase in resistance resulted when fish were immersed in T.W. to which additional sodium chloride (9 gm/l) had been added (Fig. 5B). These fish were able to withstand the highest chlorine challenge of any mosquito fish tested to date. Similarly, the explanation for the reduced toxicity of chlorinated effluent to the amphipod Gammarus may be attributable to the protective action of the elevated salt content of sewage which ranges between 25-45 ppm above carrier waters, as measured by chlorides (ECKENFELDER 1970), rather than to its nutrient levels (BRUNGS 1973). Conversely, the lack of protection by low salt levels may account for the extreme toxicity of chloramines to amphipods and to fathead minnows in the soft water (ARTHUR and EATON, 1971).

Other mortality studies (Fig. 2, 3) also indirectly suggest that the damaged gills retain only minimal ionoregulatory and coupled transport functions, in contrast to intact gills (SOMERO and HOCKACHKA 1973). This helps to explain the seeming paradox of lowered toxicities in potassium-enriched waters after intoxication. In damaged gills, ion transport mechanisms are likely to be overwhelmed, even if fully functional, because of confluence between body fluids and the external water (VALENZUELA 1976). Thus, placing the unintoxicated fish in a saline solution that is the approximate ionic equivalent of its blood (PROSSER 1973) stressed the fish and was highly toxic (Fig. 2). However, when chlorine was added to this physiological saline, toxicity was actually reduced. Similarly, experiments with equimolar concentrations of K^+, Na^+, and $Na^+ + K^+$ confirmed that chlorine reduced toxicity to potassium (Fig. 3). In a parallel series, the divalent cations Ca^{2+} and Mg^{2+} were also found to reduce toxicity to K^+ but only Mg^{2+} also reduced toxicity to chlorine (COHEN IN PREPARATION).

In TABLE II evidence is presented that intoxicated fish have an improved chance for recovery from disequilibrium, i.e., to regain normal orientation and ability to swim properly, when allowed to recuperate in an ionically and osmotically appropriate salt water before being returned to T.W. Fish suffered from osmotic stress when transferred directly from T.W. to 50% and 100% S.W.; the latter in particular proved to be quite toxic (Fig. 1) for unacclimated fish. However, the fish, which were intoxicated in T.W., rapidly recovered from disequilibrium after initial immersion in 50% and 100% S.W. (TABLE II). Despite the initial restoration of equilibrium in both these media, all fish placed in the 100% S.W. died in less than 2 hrs and those in 50% S.W. died overnight. This subsequent mortality most probably reflects osmotic stress rather than latent toxicity of chlorine. In contrast, when the fish remained in 100% S.W. for only 15-60 mins instead of 2 hrs and then placed in T.W. almost half survived overnight. Despite the demonstration of recoveries from acute intoxication, chronic experiments are necessary to establish when these moderately euryhaline fish fully regain the capacity to survive unaided in their normally wide range of waters.

Mosquito fish exhibit no diminution in oxygen consumption even when mortally intoxicated (KATZ and COHEN 1975); FOBES (1971) reported similar findings in the white sucker. Moreover, the higher resistance of mosquito fish to chlorine in brackish waters and higher rate of recovery in those media are factors that are independent of oxygen demand and are further reasons why it is unlikely that hypoxia is the primary cause of death. In addition, VALENZUELA (1976), using PAS stain, was able to demonstrate that mucus secretion did not increase in the gills of intoxicated fish and could not, therefore, clog the gills. In contrast chlorine (BASS and HEATH 1975) and other pollutants such as 3-tri-fluoromethyl-4-nitrophenol (CHRISTIE and BATTLE 1963) are believed to cause death by hypoxia in rainbow trout by eliciting a copious mucus secretion over the gills. Accordingly, a provisional explanation for differences in

species sensitivity is that sensitive species suffocate from clogged gills as a result of hypersecretory activity of mucus cells, whereas resistant species which do not hypersecrete mucus will resist chlorine intoxication until damage to gills disrupts ionoregulatory functions and osmotic barriers.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION: Standard methods for the examination of water, sewage, and industrial wastes. 11th ed. New York 1971.

ARTHUR, J. W. and J. G. EATON: J. Fish. Res. Board Can. 28, 1841-1845 (1971).

BAKER, J. T.: J. Fish. Res. Board Can. 26, 2785-2793 (1969).

BASS, M. L. and A. G. HEATH: Am. Zool. 15, 818 (1975).

BRUNGS, W. A.: J. Water Pollut. Control Fed. 45, 2180-2193 (1973).

CHRISTIE, R. M. and H. I. BATTLE: Can. J. Zool. 41, 51-64 (1963).

DANDY, J. W. T.: Can. J. Zool. 50, 405-410 (1972).

ECKENFELDER, W. W.: Water quality engineering for practicing engineers. New York, Barnes and Noble. 1970. Pg. 58.

FOBES, R. L.: Chlorine toxicity and its effect on gill tissue respiration of the white sucker Catostomus commersoni (Lacepede). Master's thesis, Michigan State Univ., East Lansing 1971.

HOCHACHKA, P. W. and G. N. SOMERO: Strategies of biochemical adaption. Phila., W. B. Saunders Co. 1973. Pgs. 125-135.

KATZ, B. M. and G. M. COHEN: Q. J. Fla. Acad. Sci. 38 (Suppl. 1), 12 (1975).

KATZ, B. M. and G. M. COHEN: Bull. Environ. Contam. Toxicol. 15, 644-650 (1976)

PENZES, B.: Biological Abstracts 53, #34643 (1972).

PROSSER, C. L.: Comparative animal physiology, 3rd ed. Phila., W. B. Saunders Co. 1973. Pg. 87.

SCHMID, O. J. and H. MANN: Nature 192, 675 (1961).

VALENZUELA, J. M.: A fine structural examination of the gill in the mosquito fish, Gambusia affinis, after exposure to chlorine. Master's thesis, Florida Institute of Technology, Melbourne 1976.