

# FRESHWATER TO SALTWATER TOXICITY EXTRAPOLATION USING SPECIES SENSITIVITY DISTRIBUTIONS

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**Abstract**—There is generally a lack of saltwater ecotoxicity data for risk assessment purposes, leaving an unknown margin of uncertainty in saltwater assessments that utilize surrogate freshwater data. Consequently, a need for sound scientific advice on the suitability of using freshwater data to extrapolate to saltwater effects exists. Here we use species sensitivity distributions to determine if freshwater datasets are adequately protective of saltwater species assemblages for 21 chemical substances. For ammonia and the metal compounds among these data, freshwater data were generally protective because freshwater organisms tended to be more sensitive. In contrast, for pesticide and narcotic compounds, saltwater species tended to be more sensitive and a suitable uncertainty factor would need to be applied to surrogate freshwater data. Biological and physicochemical factors contribute to such differences in freshwater and saltwater species sensitivities, but the species compositions of datasets used are also important.

Keywords—Saltwater data Risk assessment Species sensitivity distributions

## **INTRODUCTION**

Insufficient toxicity data often exists for saltwater organisms for European environmental regulators to estimate a saltwater predicted-no-effect concentration (PNEC) with confidence. This is because fewer standard test methods for saltwater species have been developed and because aquatic risk assessments have traditionally tended to focus on freshwater systems. Freshwater toxicity data are therefore usually more plentiful and their use may provide a suitable surrogate for saltwater data. Currently, the relationship between the sensitivity to toxicants of saltwater and freshwater organisms is not well understood [1], and risk assessments tend to assume that freshwater species respond similarly to saltwater species. These assumptions remain untested and have led to proposals to add an additional safety factor of 10 to saltwater risk assessments based on freshwater data (S. Robertson, Environment Agency of England and Wales, personal communication). Here we deal explicitly with the European perspective [2]; however, we recognize differences between the United States [3] (http://www.tnrcc.state.tx.us/permitting/waterperm/wqstand/ wer\_45.pdf) and Australian regulatory approaches [4].

In this article, we utilize a widely available aquatic toxicity database to compare species sensitivity distributions (SSDs) for saltwater and freshwater organisms exposed to the same chemicals, following the method described by Leung et al. [5]. The first objective is to identify chemical groups, classified by mode of action or physicochemical properties, where freshwater and saltwater SSDs effectively coincide. Here, technically sound estimates of saltwater PNECs may be made on the basis of freshwater toxicity data. The second objective is to consider the species composition of a toxicity dataset required to estimate saltwater PNECs with confidence and assess the risks of drawing false conclusions associated with a surrogate approach to estimate a saltwater PNEC.

## MATERIALS AND METHODS

Acute freshwater and saltwater median lethality data (LC50) for 21 substances were extracted from the U.S. Environmental Protection Agency AQUIRE aquatic toxicology database (http://www.epa.gov/ecotox/).

Toxicity-effects data were ranked and assigned percentiles. The data were then fitted with the two distributions commonly used to construct SSDs, the linearized log-normal approach of Wagner and Løkke [6] and the log-logistic approach developed by Aldenberg and Slob [7], although we recognize that other nonparametric approaches are available [8]. Multiple data for the same species were summarized as geometric means [9]. Both techniques were used to estimate the hazardous concentration for 5% of species (HC5), or the 95% protection level [10] with its associated lower 95% confidence intervals (one tailed). Log-logistic  $\alpha$  (location parameter) and  $\beta$  (scatter parameter) and log-normal slope and *y*-intercept regression coefficients were also calculated.

Comparisons between freshwater and saltwater datasets were based on HC5 and regression parameter values [5]. Plots were visually inspected on the same axes to establish the extent to which freshwater and saltwater distributions coincided. For the log-normal transformed data, analyses of covariance (AN-COVA; SPSS, Chicago, IL, USA) were carried out to compare the slopes and intercepts resulting from the SSDs [11]. The percentage taxonomic composition of each SSD was calculated and expressed in pie charts.

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Table 1. Comparison of the freshwater and saltwater species sensitivity distributions using the log-logistic model,  $y = 1/[1 + \exp(-(x - \alpha)/\beta];$ hazardous concentration for 5% of species (HC5) values expressed as  $\mu g/L$ ; CL is the one-sided left confidence limit

			Fresh	water		Salt water							
Substance	α	β	$r^2$	HC5	CL	α	β	$r^2$	HC5	CL			
Ammonia	3.610	0.493	0.839	143.6	24.07	4.460	0.402	0.979	1,892	442.4			
Cadmium	2.960	0.706	0.966	7.60	1.08	3.083	0.737	0.940	8.22	1.07			
Copper	2.368	0.559	0.967	5.27	1.14	2.333	0.332	0.971	22.7	6.84			
Lead	3.792	0.588	0.969	115.1	35.7	3.820	0.490	0.901	238.2	62.0			
Mercury	2.242	0.634	0.947	2.38	0.145	2.223	0.443	0.921	8.30	0.942			
Nickel	3.438	0.567	0.942	58.6	2.49	4.093	0.465	0.970	529.7	25.2			
Potassium dichromate	4.153	0.529	0.959	394.0	137.4	4.178	0.324	0.942	1,675	691.0			
Zinc	3.284	0.602	0.980	32.4	3.68	3.513	0.297	0.966	435.0	93.0			
Chlordane	1.760	0.429	0.918	3.14	0.664	0.837	0.367	0.947	0.570	0.039			
Chlorpyrifos	0.928	0.676	0.966	0.086	0.023	0.872	0.863	0.886	0.021	0.001			
Dieldrin	1.355	0.497	0.965	0.780	0.290	1.458	0.525	0.913	0.817	0.060			
Endosulfan	1.231	0.751	0.886	0.105	0.024	0.604	0.830	0.897	0.014	0.001			
Lindane	2.309	0.578	0.979	4.05	1.28	1.909	0.738	0.914	0.545	0.072			
Malathion	2.767	0.759	0.963	3.40	1.21	2.287	0.679	0.955	1.94	0.167			
Benzene	4.893	0.287	0.962	11,169	3,959	5.072	0.330	0.906	12,589	522.2			
Dichloroaniline	3.712	0.342	0.961	507.0	103.3	3.717	0.270	0.896	835.6	184.8			
Pentachlorophenol	2.795	0.454	0.929	28.7	11.6	2.767	0.358	0.973	51.5	19.3			
Phenol	4.932	0.415	0.960	5,129	2,904	4.549	0.360	0.977	3,083	834.8			
Thiobencarb	3.379	0.287	0.978	342.0	121.6	2.642	0.143	0.927	166.3	42.1			
Toluene	4.838	0.306	0.973	8,650	2,237	4.767	0.386	0.887	4,271	180.2			
Trichloroethane	4.963	0.118	0.908	41,257	15,585	4.831	0.177	0.771	20,417	8,110			

#### RESULTS

The HC5 and regression parameters for both saltwater and freshwater distributions are summarized in Table 1 (log logistic) and Table 2 (log normal). Comparisons based on AN-COVA are reported in Table 3. Results for ammonia, metals, pesticides, and narcotics are discussed below. The distributions are based on a range of species numbers (data points) from 7 to 150 for freshwater and 6 to 36 for saltwater datasets (Table 2).

## Ammonia

A clear difference between freshwater and saltwater SSDs exists (Fig. 1), with markedly greater sensitivity exhibited by freshwater species. This is reflected in an order-of-magnitude difference in HC5 values (Tables 1 and 2) and significant differences in *y*-intercepts (Table 3). However, the freshwater model fit was rather poor ( $r^2$ : log logistic, 0.84; log normal 0.83). The freshwater dataset is well populated with fish (34%) and crustacean (26%) data, while the saltwater dataset is dominated by crustaceans (72%). Data for saltwater annelid or platyhelminth species are absent.

# Metals

*Cadmium.* A high degree of congruence exists between freshwater and saltwater SSDs, with very similar HC5 estimates (Fig. 2a, Tables 1 and 2) indicating close agreement between the sensitivity to cadmium of saltwater and freshwater assemblages. Despite this, ANCOVA indicates that the *y*-intercepts are statistically significantly different. Conclusions are based on reasonably well-populated freshwater and saltwater datasets dominated by crustaceans (27 and 46%, respectively) and fishes (19 and 42%, respectively). The saltwater dataset has a higher proportion of fish and crustacean data, largely as a result of the increased taxonomic diversity of the freshwater dataset, which also includes insects, annelids, platyhelminthes, and ectoprocts, all of which are lacking in the saltwater dataset.

*Copper*. Some congruence is found between freshwater and saltwater SSDs (Fig. 2b), although ANCOVA highlights sig-

nificant differences in slope parameters (Table 3), resulting in greater sensitivity of freshwater organisms when compared with saltwater organisms in the lower tails of the distributions. This is reflected in lower freshwater than saltwater HC5 values, differing by approximately fivefold for both models. The freshwater dataset represents a wide taxonomic range (10 taxa), with fishes (37%) and crustaceans (29%) being the best represented. While not containing as many taxa (partly because insects and amphibians are absent), the saltwater dataset also represents reasonable taxonomic coverage. Freshwater data are likely to be adequately protective for saltwater organisms by a substantial margin.

*Lead.* Freshwater and saltwater SSDs produce similar slope and HC5 parameters with both models (Tables 1 and 2), although model fits, particularly for the more tolerant species, are not as good as for other substances (Fig. 2c). Freshwater biota are more sensitive, though not by a large degree, but use of freshwater data would still be adequately protective of saltwater organisms. Crustaceans are well represented in both freshwater (46%) and saltwater (50%) datasets. However, freshwater mollusk and insect and saltwater annelid data are not represented in the datasets.

*Mercury*. Relatively small datasets for mercury give rise to SSDs with significantly different slopes (Fig. 2d, Table 3). Nevertheless, the HC5 values for freshwater and saltwater species differ by only around fourfold using either model (Tables 1 and 2), with greater sensitivity exhibited by freshwater species. Fishes are equally well represented in freshwater and saltwater datasets (39 and 38%, respectively), as are mollusks (7 and 8%, respectively). Insects, which represent 26% of species in the freshwater data, are lacking from the saltwater set, in which crustaceans represent 46% of species (7% in freshwater). Freshwater data are again likely to be adequately protective of saltwater species.

*Nickel.* Among the metals, nickel shows the largest difference between freshwater and saltwater data (Fig. 2e), with greater sensitivity of freshwater species. No evidence of a difference in slope of the two distributions is found (Table 3); Table 2. Comparison of the freshwater and saltwater species sensitivity distributions using the log-normal model, y = ax + b; hazardous concentration for 5% of species (HC5) values expressed as  $\mu g/L$ ; CL is the one-sided left confidence limit; *n* is the number of species and data points

I			Fr	eshwater						Salt water		
Substance	и	a	p	$r^2$	HC5	CL	и	a	p	$r^2$	HC5	CL
Ammonia	27	0.927	-3.348	0.830	68.58	10.28	14	1.162	-5.185	0.964	1,110	529.2
Cadmium	42	0.723	-2.140	0.986	4.836	2.564	31	0.656	-2.022	0.913	3.763	0.608
Copper	42	0.898	-2.126	0.949	3.434	1.273	24	1.490	-3.477	0.984	16.96	12.06
Lead	99	0.884	-3.353	0.886	85.49	45.79	36	0.977	-3.733	0.904	137.1	32.39
Nickel	11	0.789	-2.7114	0.929	22.52	4.045	6	0.962	-3.936	0.979	241.3	109.1
Mercury	15	0.745	-1.669	0.971	1.078	0.393	13	1.042	-2.315	0.954	4.401	1.688
Potassium dichromate	79	0.968	-4.019	0.938	284.0	105.9	33	1.541	-6.437	0.961	1,288	772.0
Zinc	28	0.826	-2.712	0.974	19.60	8.958	12	1.555	-5.461	0.971	285.1	170.7
Chlordane	25	1.111	-1.955	0.910	1.903	0.617	×	1.177	-0.985	0.945	0.275	0.084
Chlorpyrifos	90	0.772	-0.716	0.965	0.063	0.025	19	0.537	-0.468	0.898	0.0064	0.0005
Dieldrin	58	1.030	-1.396	0.959	0.573	0.267	33	0.893	-1.302	0.847	0.412	0.068
Endosulfan	76	0.662	-0.815	0.886	0.056	0.008	25	0.572	-0.346	0.904	0.005	0.001
Lindane	76	0.912	-2.105	0.983	3.196	1.853	35	0.661	-1.262	0.914	0.263	0.044
Malathion	150	0.686	-1.914	0.974	2.472	1.036	28	0.716	-1.638	0.932	0.979	0.222
Benzene	28	1.741	-8.518	0.983	8,870	6,577	9	1.263	-6.403	0.958	5,876	1,780
Dichloroaniline	14	1.346	-4.995	0.934	308.5	126.6	11	1.634	-6.073	0.907	513.0	193.6
Pentachlorophenol	80	1.114	-3.113	0.915	20.80	7.620	30	1.399	-3.871	0.980	38.99	26.21
Phenol	143	1.278	-6.301	0.977	4,409	2,804	28	1.356	-6.167	0.941	2,166	1,046
Thiobencarb	28	1.709	-5.773	0.945	260.8	149.4	9	2.863	-7.562	0.920	116.7	52.07
Toluene	18	1.571	-7.599	0.975	6,176	4,027	7	1.121	-5.344	0.981	1,994	961.3
Trichloroethane	7	3.626	-18.00	0.965	32,282	23,378	12	2.424	-11.71	0.841	14,206	5,897

Table 3.	Analysis	of	covariance	comparisons	of	the	slopes	and	y-intercepts	for	log-normal	freshwater	and	saltwater	species	sensitive
							dis	strib	utions (SSDs)							

	Difference in	n slopes	Difference in y-intercept <sup>a</sup>				
Substance	F value	p Value	F value	p Value			
Ammonia	$F_{1,37} = 2.63$	0.113	$F_{1,37} = 185.0$	< 0.001			
Benzene	$F_{1,30} = 19.4$	< 0.001	2, <i>21</i>				
Cadmium	$F_{1,69} = 3.41$	0.069	$F_{1,69} = 1,223$	< 0.001			
Chlordane	$F_{1,29} = 9.39$	0.005	1, 07				
Chlorpyrifos	$F_{1,105} = 44.3$	< 0.001					
Copper	$F_{1,62} = 70.8$	< 0.001					
Dichloroaniline	$F_{1,21}^{1,02} = 2.17$	0.156	$F_{1,21} = 227.3$	< 0.001			
Dieldrin	$F_{1,87}^{(1,21)} = 4.69$	0.033	., 2.				
Endosulfan	$F_{1,97} = 3.15$	0.079	$F_{1,97} = 190.0$	< 0.001			
Lead	$F_{1.98} = 3.50$	0.064	$F_{1,98} = 1,318$	< 0.001			
Lindane	$F_{1,128} = 77.1$	< 0.001	-, , , ,				
Malathion	$F_{1,174} = 1.14$	0.287	$F_{1,174} = 2,208$	< 0.001			
Mercury	$F_{1,24} = 16.3$	< 0.001					
Nickel	$F_{1, 16} = 2.87$	0.109	$F_{1, 16} = 269.1$	< 0.001			
Pentachlorophenol	$F_{1,106} = 13.0$	< 0.001	-, - •				
Phenol	$F_{1,167} = 2.30$	0.131	$F_{1,167} = 2,723$	< 0.001			
Potassium dichromate	$F_{1,108} = 61.4$	< 0.001	-,				
Thiobencarb	$F_{1,30} = 8.68$	0.006					
Toluene	$F_{1,21} = 19.9$	< 0.001					
Trichloroethane	$F_{1,15} = 3.47$	0.082	$F_{1, 15} = 86.6$	< 0.001			
Zinc	$F_{1, 36} = 68.2$	< 0.001	, .				

<sup>a</sup> Comparisons of *y*-intercepts are only made when there are no significant differences between slopes [8].

rather, the distribution for freshwater species is shifted toward lower effect concentrations, resulting in an order of magnitude difference in HC5 values. The relative proportions of fish and crustaceans are split between freshwater (46 and 18%, respectively) and salt water (22 and 56%, respectively).

*Potassium dichromate.* Analysis of covariance shows a significant difference in freshwater and saltwater slope parameters (Fig. 2f, Table 3). The more sensitive freshwater species are affected to a greater extent than the most sensitive saltwater species (Tables 1 and 2). The HC5 values reflect the greater sensitivity of the freshwater species compared with the saltwater species. Fishes, crustaceans, and (unusually) annelids are well represented in both saltwater and freshwater datasets, but very few saltwater mollusk data exist. The freshwater species composition is affected by the inclusion of one study on 12 species of protozoa, although they probably had little influence on the outcome because they exhibited intermediate sensitivity to this substance.

Zinc. The ANCOVA shows a significant difference in freshwater and saltwater slope parameters (Fig. 2g, Table 3). Once again, freshwater species are generally more sensitive than



Fig. 1. Freshwater and saltwater species sensitivity distributions (SSDs) for ammonia. Solid symbols indicate freshwater data points, open symbols indicate saltwater data points. Pie charts represent the taxonomic composition of the distributions; upper freshwater (FW) and lower salt water (SW).

saltwater species (Tables 1 and 2). The HC5 values reflect this sensitivity, with more than an order of magnitude separating freshwater and saltwater species. Despite a lack of data for insects, amphibians, and ectoprocts in the saltwater dataset, the taxonomic compositions are broadly similar. Fishes are well represented in both freshwater (35%) and saltwater (18%) sets, as are crustaceans (28 and 55%, respectively), but data for freshwater insects are sparse.

## Pesticides

*Chlordane.* Displacement of the freshwater and saltwater SSDs (Fig. 3a) indicates greater sensitivity of saltwater species, which is also shown by differences in HC5 estimates for both the log-logistic and log-normal models (Tables 1 and 2). The ANCOVA also indicates significant differences in the slopes (Table 3). The freshwater dataset contains high proportions of fish (60%) and crustacean (24%) data, but meaningful comparison with the saltwater dataset is difficult because of the small size (8 species) of the latter. These results suggest that freshwater data are not necessarily protective of saltwater organisms exposed to chlordane.

*Chlorpyrifos.* Very close agreement is found of the two SSDs (Fig. 3b), both of which are well populated with data. However, ANCOVA indicates a difference in slope (Table 3). Differences in HC5 estimates are evident with both fitted models, with a 10-fold difference using the log-normal model (Table 1) and a 5-fold greater sensitivity for saltwater species using the log-logistic model (Table 2). A taxonomic analysis of the datasets reveals a high proportion of freshwater insect data (56%), which is perhaps not surprising because this chemical is an insecticide. Fishes (23%) and crustaceans (17%) are also well represented. Fishes (53%) represent a large proportion of the saltwater dataset, as do crustaceans (32%). Some data for salt-tolerant dipterans (5%) exist. Neither dataset has few mollusk data, although these are unlikely to be par-

Freshwater to saltwater toxicity extrapolation



Fig. 2. Freshwater (FW) and saltwater (SW) species sensitivity distributions (SSDs) for metals. Pie charts represent the taxonomic composition of the distributions (upper freshwater and lower salt water). Conventions as for Figure 1.



Fig. 3. Freshwater (FW) and saltwater (SW) species sensitivity distributions (SSDs) for pesticides. Pie charts represent the taxonomic composition of the distributions (upper freshwater and lower salt water). Conventions as for Figure 1.

ticularly sensitive. Freshwater data are not necessarily protective of saltwater organisms exposed to chlorpyrifos.

*Dieldrin*. Despite a difference in slope (Fig. 3c, Table 3), good congruence occurs between the distributions, as confirmed by similar HC5 values from both model fits (Tables 1 and 2). Insects (36%) are well represented in the freshwater dataset, as are fishes (40%) and, to a lesser extent, crustaceans (14%). Considerable amounts of data for saltwater fish species (52%) also exist, and saltwater crustaceans (30%) are also well represented. Relatively few freshwater mollusk data can be found. Freshwater data are likely to be adequately protective of saltwater species.

Endosulfan. Despite poor model fits to both the freshwater and saltwater data (Fig. 3d), indications are, from the saltwater SSD, that a greater sensitivity to endosulfan exists. The HC5 values indicate a difference approaching an order of magnitude (Tables 1 and 2). Although both datasets are well populated with data, the taxonomic composition of each is quite different, and this could contribute to the observed difference in sensitivity. Fishes are well represented in both freshwater and saltwater datasets (62 and 36%, respectively), as are crustaceans (17 and 40%, respectively). Mollusks (13%) are the next best represented taxon in freshwater, while annelids are better represented in salt water (12%). Freshwater annelid data do not exist, and relatively little saltwater mollusk data is found. The much better representation of crustaceans in the saltwater dataset may be significant because effect concentrations for these species tend to occur in the lower tail of the saltwater SSD (the first, fourth, fifth, and seventh most sensitive saltwater species are all crustaceans). These data suggest that freshwater data are not necessarily protective of saltwater organisms exposed to endosulfan.

*Lindane.* Some indications exist of a displacement of the saltwater SSD toward lower effect concentrations (Fig. 3e), especially for species that exhibit intermediate sensitivity to lindane. This is reflected in the HC5 values estimated by both models (Tables 1 and 2), which show a difference of around an order of magnitude. The difference in the number of taxa represented is largely due to the lack of insects and amphibians in the saltwater dataset. The proportion of fishes in the two datasets is identical (46%), and proportions of arthropods are very similar (38% in freshwater, 37% crustaceans in salt water). Saltwater annelid and platyhelminth data are lacking. These data suggest that freshwater data are not necessarily protective of saltwater organisms exposed to lindane.

*Malathion.* Despite a lack of congruence among species showing greater tolerance to malathion, better agreement between the SSDs is evident among the more sensitive species (Fig. 3f). This leads to only a modest difference in HC5 (Tables 1 and 2), with saltwater species tending to be more sensitive. Fishes represent 36% of the freshwater data and 39% of the saltwater data, while arthropods represent 44% of data in freshwater and 50% in salt water. Large amounts of data for freshwater mollusks are available but few exist for saltwater species. Data for saltwater annelids and platyhelminthes are again completely lacking. These data suggest that freshwater data may not be protective of saltwater organisms exposed to malathion, although differences are smaller than for some other pesticides.

*Thiobencarb.* The disparity between number of taxa and species makes it difficult to draw meaningful conclusions for this herbicide (Fig. 3g). Nevertheless, a consistent tendency toward greater sensitivity of saltwater species exists, with a twofold difference using either model. Both freshwater and

saltwater data contain fishes (46 and 67%, respectively) and crustaceans (21 and 33%, respectively), but annelid and platyhelminth data are lacking. Saltwater rotifer and mollusk data are also lacking. Perhaps most significantly for a herbicide, data for algae are sparse. These data suggest that freshwater data may not be protective of saltwater organisms exposed to thiobencarb, although, as for malathion, differences are smaller than for some other pesticides.

# Narcotics

*Benzene.* Good congruence between the SSDs is evident, particularly in the lower tails of the distributions (Fig. 4a). This is reflected in similar HC5 values (Tables 1 and 2), although ANCOVA shows significant differences in slope (Table 3). The freshwater dataset is well populated with fish (28%), crustacean (25%), and insect (21%) data, but the saltwater set only comprises six species, with annelids and platyhelminths absent. Despite this, on the basis of the data here, it is reasonable to conclude that freshwater data are likely to be adequately protective of saltwater organisms.

Dichloroaniline. The SSDs for freshwater and saltwater organisms are congruent (Fig. 4b), although significant differences in y-intercepts are indicated by ANCOVA (Table 3). The differences in HC5 values are relatively small for both models (Tables 1 and 2). Apart from a lack of insects in the saltwater dataset, considerable agreement is found in the freshwater and saltwater taxonomic coverage. Fishes and crustaceans are the dominant taxa in both datasets, with mollusks (14% in both cases) also well represented. One species of rotifer and one species of annelid are found in both distributions. Although saltwater species, the differences are sufficiently small that data on the latter are likely to protect the former within current European risk assessment frameworks.

*Pentachlorophenol.* Although ANCOVA indicates a statistically significant difference between the slopes of the fitted lines (Table 3), the freshwater and saltwater SSDs are visually congruent (Fig. 4c). The HC5 estimates differ by less than a factor of two with both model fits (Tables 1 and 2), with saltwater species being more sensitive than freshwater species. Both freshwater and saltwater datasets are well populated with data for fishes (30 and 20%, respectively), arthropods (42 and 38%, respectively), and mollusks (13 and 23%, respectively). Annelids and platyhelminthes are represented in both datasets, but there is an absence of saltwater rotifer data. As for dichloroaniline, although saltwater species are sufficiently small that data on the latter are likely to protect the former within current European risk assessment frameworks.

*Phenol.* Differences in the distributions are evident in the upper tails (i.e., the more tolerant species), with greater sensitivity exhibited by saltwater species (Fig. 4d). However, greater congruence is evident among the more sensitive species. The HC5 values are up to twofold different, depending on the model fitted (Tables 1 and 2), with saltwater species showing lower sensitivity. The major taxa are present in both freshwater and saltwater datasets, although saltwater annelid and platyhelminth data are lacking. Despite this, freshwater data are likely to be adequately protective of saltwater organisms.

*Toluene*. Broadly similar SSDs emerge from the available data (Fig. 4e), although a tendency toward greater sensitivity for saltwater species in the lower tails of the distributions fitted



Fig. 4. Freshwater (FW) and saltwater (SW) species sensitivity distributions (SSDs) for narcotics. Pie charts represent the taxonomic species composition of the distributions (upper freshwater and lower salt water). Conventions as for Figure 1.

by both models exists (Tables 1 and 2). Fish and crustacean data dominate both datasets, and no annelid or platyhelminth data are found in either. Freshwater data could not be used with great confidence to protect saltwater species exposed to toluene.

*Trichloroethane.* The least toxic of all the compounds analyzed, SSDs fitted to trichloroethane are similar to those for toluene (Fig. 4f). Rather poor model fits to the data are evident (Tables 1 and 2), but lower sensitivity is exhibited by saltwater species for both model fits. In contrast with other chemicals analyzed in this article, more species are included in the saltwater than in the freshwater dataset, although numbers for both are small. Fish are represented in both freshwater (43%) and saltwater (25%) datasets, as are mollusks (29 and 17%, respectively) and arthropods (28 and 41%, respectively). Data for rotifers are lacking for both media.

## DISCUSSION

The degree of correlation between freshwater and saltwater species is influenced by the three main factors of biological differences between saltwater and freshwater organisms; chemical differences in each medium, especially speciation and bioavailability; and methodological differences in tests that could lead to systematic differences in toxicity estimates.

An understanding of these factors is vital in any risk assessment using freshwater data as surrogates for the responses of saltwater species. Evidence is presented in this article that the differences between freshwater and saltwater responses, as described by species sensitivity distributions, are generally not great. For ammonia and the metals that were investigated, freshwater species are more sensitive, and use of freshwater data would be protective of saltwater species. In contrast, saltwater species are generally more sensitive to pesticides and narcotics and would not be protected by use of freshwater toxicity data. However, differences in toxicity are generally not large and a modest safety factor may be sufficient to account for them.

Freshwater species were markedly more sensitive than saltwater species to ammonia. This could be associated with differences in the taxonomic composition of the two datasets, i.e., the freshwater set was well populated with fish and crustacean data whereas the saltwater dataset was dominated by crustaceans. The preponderance of freshwater fish data is significant because fishes are generally regarded as being particularly sensitive to ammonia, and indeed, they dominated the tail of the freshwater SSD. This may account for the greater sensitivity of freshwater biota but could only be confirmed by the addition of data for saltwater fish species.

All seven metals investigated were more toxic under freshwater conditions than under saltwater conditions, although to different extents. Based on HC5 values, the greatest difference was seen with zinc (a 13-fold difference). The different sensitivities of freshwater and saltwater organisms is likely to be, in part, a consequence of differences in speciation and bioavailability for the different media and, in particular, the greater abundance of uncomplexed or free ionic forms of metals under freshwater conditions [12,13]. In addition, the greater ability of saltwater organisms to regulate uptake of zinc and copper is also likely to contribute to greater tolerance of these substances by such organisms [14]. It has not been possible to determine whether or not SSDs tend to coincide when toxicity is expressed in terms of the bioavailable form(s) of the metal because this information is not usually reported. However, for the purpose of metals risk assessment, this is an academic point because we can be reasonably confident that conservative saltwater assessments could be based on freshwater toxicity data without a safety factor.

Differences in solubility (and chemical activity) of lipophilic compounds between saltwater and freshwater media are probably due to a salting out phenomenon. Higher salinities effectively squeeze out neutral organic molecules due to the strong ionic interactions among water molecules and the major seawater ions, resulting in reduced solubility in salt water. At levels below saturation, this means that the effective concentration of the substance is higher, leading to increased activity and greater bioavailability. This is not a particular feature of these compounds' mode of toxic action but rather a consequence of their physicochemical properties. If this is true, then a greater possibility of risk to saltwater organisms exists, which will need to be considered in a risk assessment. Alternatively, one could hypothesize that, as crustaceans (which will often be as sensitive as insects to insecticides) comprise a dominant part of the saltwater dataset, they may have introduced a bias toward greater saltwater sensitivity to the insecticides, especially if insects and crustaceans are not well represented in the freshwater dataset. Closer examination of the datasets for chlorpyrifos, lindane, and malathion shows good representation in both freshwater and saltwater datasets of insects and/ or crustaceans and, moreover, these were invariably the most sensitive five or six species of those tested. For these substances, at least, this explanation is not compelling. However, for endosulfan, fishes dominated the freshwater dataset (and the lower tail of the freshwater SSD) while crustaceans occupied this role in the saltwater distribution. For endosulfan, apparently greater sensitivity by saltwater organisms may thus be overestimated due to inadequate representation of crustaceans and/or insects in the freshwater dataset. This could be investigated by reinforcing the freshwater endosulfan datasets with crustacean (or insect) data.

For saltwater risk assessments, close examination of the species composition of the freshwater dataset is warranted before deciding whether or not an additional safety factor needs to be added to account for possibly greater sensitivity of saltwater biota. For insecticides, insect and crustacean taxa need to be well represented, while for herbicides, the presence of data for algae and higher plants will be key. On the basis of the data reported in this article, it seems that a modest safety factor should be applied if freshwater data are used to protect saltwater organisms from pesticide exposure.

Of the narcotics investigated, only phenol may be regarded as a polar narcotic (log  $K_{ow} = 1.51$ ); the others are examples of nonpolar narcotics (log  $K_{ow} = 2.0-2.54$ ). Again, there was a modest tendency toward greater toxicity to saltwater organisms, although in some cases this conclusion is based on rather small datasets. Given the nonspecific mode of action of these compounds, we would not expect a priori any particular taxonomic group to be more or less sensitive than another (except perhaps as a consequence of body size [15]). Thus, the most plausible explanation for this consistent trend is a salting out phenomenon [16].

A study reported by Zaroogian et al. [17] is useful because it allows us to examine the ecotoxicological consequences of such differences in solubility. They developed quantitative structure-activity relationships for neutral organic compounds and the mysid shrimp (Americamysis bahia). Toxicity was consistently underpredicted when predicted toxicities, based on freshwater solubilities, were compared with experimentally derived values. For toluene and tetrachloroethylene (where the difference in solubility was smallest), the solubility adjustment did not result in a bias toward the experimental data. However, when solubility data corrected for seawater ionic strength were applied in the Zaroogian et al. [17] algorithms used to predict toxicity, the discrepancy was narrowed, at least for six of the eight chemicals. Despite this, differences between predicted and measured LC50 values were greater than could be accounted for solely by the influence of salinity on solubility. It is not possible to identify the precise reason for these differences, although it is clear that different solubilities in freshwater and salt water could go some way toward explaining differences in SSDs for narcotic compounds.

In practice, a lipophilic test substance will not be in equilibrium between the test medium and test organisms for much (possibly all) of the duration of an acute toxicity study. Thus, it is possible that kinetic factors play a part when the time taken to reach a critical body burden [18] exceeds the duration of the toxicity study [19]. It is possible that an increase in activity of a toxicant (such as would arise due to an increase in salinity) leads to a greater likelihood of the critical body burden being reached within the duration of an acute toxicity study, with consequent differences in the expression of toxicity in freshwater and saline media. If so, it follows that the differences between freshwater and saltwater SSDs reported here might not be apparent when based on chronic exposure data, where critical body burdens are more likely to be achieved within the period of exposure.

In saltwater risk assessments, the greater sensitivity of saltwater organisms to compounds with a narcotic mode of action may need to be considered. The difference between freshwater and saltwater HC5 values was actually rather consistent and never more than a factor of two (Tables 1 and 2). Therefore, for risk assessment purposes, only a small safety factor should be applied when using freshwater data to protect saltwater species from exposure to narcotic chemicals. However, this conclusion needs to be qualified in view of small datasets and especially the lack of data for polar narcotics. It is also noteworthy that the difference, although small, is a consistent one. For industrial chemicals, the narcotic mode of action predominates, and so we would advise the generation of additional saltwater toxicity data for further polar narcotic compounds to validate this conclusion. Attention to the relationship between freshwater and saltwater SSDs based on chronic toxicity data is also warranted.

The results for ammonia, metals, pesticides, and narcotics found in this study can be summarized by ranking the freshwater to saltwater HC5 ratios for each substance and plotting percent ranks against this ratio (Fig. 5). Equivalent sensitivities are found along the zero line (log scale); those substances falling to the left indicate greater freshwater sensitivity and to the right greater saltwater sensitivity. Data for the 21 substances closely follow a log-normal distribution (y = 46.47



Fig. 5. Relationship between freshwater (FW)/saltwater (SW) loglogistic hazardous concentration for 5% of species (HC5) ratios and chemical class. FW = freshwater; SW = salt water.

 $\log_{10}x + 55.05$ ;  $r^2 = 0.98$ ). It is also possible to infer a safety factor, at least for the acute data presented here, if freshwater data were used to be protective of saltwater species. Figure 5 shows the 90th percentile, identifying a factor that should protect saltwater biota based on freshwater toxicity data in 90% of cases. These values compare with Hutchinson et al. [1], who, using median effective concentration (EC50) data, found that sensitivities were within a factor of 10 for fishes (91% of substances studied) and invertebrates (33% of substances studied). With the limited data available in our datasets, a factor of approximately 5.5 would be required. However, considering the differences between chemical groups, even this small factor would be overprotective in 90% of circumstances. Therefore, a flexible approach to when to apply such a factor could be used.

In summery, a sound basis exists for using freshwater toxicity data to extrapolate to saltwater effects. The results of this study provide a useful guide to help establish the magnitude of an appropriate assessment factor to be applied to freshwater data. However, further studies with complete datasets (in terms of size and taxonomic composition) will be necessary to understand fully the differences between freshwater and saltwater responses. This will undoubtedly require saltwater data generation and further development of test methods with saltwater species. In the meantime, it is clear that comparisons made with SSDs provide an effective tool for indicating how appropriate extrapolation will be.

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