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Acute Toxicity of 3-Chloro-4-Methyl Benzenamine Hydrochloride to Shrimp and Crabs

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3-Chloro-4-methyl benzenamine HCl (DRC-1339, 3-Chloro-p-toluidine HCl, CPTH, Starlicide^{R}) is an avian toxicant currently registered to control starlings at cattle and poultry feed lots. CPTH is also highly toxic to many other speices of pest birds and is relatively non-toxic to most raptors and mammals (DeCINO et al. 1966). In 1969, the Massachusetts Audobon Society and the U.S. Fish and Wildlife Service began a cooperative study to investigate a means of controlling herring (Larus argentatus) and great black-backed (Larus marinus) gulls that were rapidly displacing terns and other seabirds from breeding islands along the northeastern U.S. coast. As a result of a five-year study, it was determined that CPTH could be used to selectively bait the encroaching gulls, thereby allowing the beseiged seabird colonies to recover. In 1975, the Fish and Wildlife Service petitioned the Environmental Protection Agency (EPA) for registration of CPTH for controlling herring and great-backed gulls on breeding islands along the U.S. northeastern coast. This study represents part of the data that were required by EPA which ultimately resulted in the registration of CPTH.

MATERIALS AND METHODS

Test Animals: Shrimp

A total of 150 penaeid shrimp (19% pink shrimp, Penaeus duorarum and 81% white shrimp, P. setiferus) was employed in this investigation. All shrimp were purchased from a local live bait dealer and ranged in length from 76 to 132 mm. Shrimp were acclimated prior to testing for one week in wading pools containing 15 ppt artificial seawater (Rila Marine Mix, Rila Products) and a substrate of washed blasting sand. Shrimp were aerated throughout the acclimation period and fed until 48 h prior to testing. Shrimp selected for testing appeared healthy and free of disease or injury. Water temperature during acclimation and testing was 20-24 C.

Test Animals: Crabs

A total of 225 blue crabs, Callinectes sapidus, was used. All crabs were collected locally by netting and ranged in width from 19-82 mm. The acclimation procedure for crabs was identical to that described above for shrimp.

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Containers: One-gallon (3.785 L) glass wide-mouth jars were used for all bioassay tests. Bioassay water was synthetic seawater (Rila Marine Mix) of 15 ppt salinity, and the quantity was 2 L in all cases.

Toxicant: The toxicant used in these bioassay evaluations was Compound DRC-1339 Starling Toxicant (CPTH) containing 95% active ingredient. Toxicant was supplied by U.S. Department of the Interior, Bureau of Sport Fisheries and Wildlife, Division of Wildlife Services.

Testing: Shrimp and crabs were randomly placed in the test vessels containing 2 L of 15 ppt artificial seawater approximately 24 h prior to addition of toxicant. Test animals were placed in test containers on a one animal per container basis to eliminate antagonism and cannibalism. Aeration was accomplished by passing a fine stream of line air through glass disposable pipets. Containers were continuously lighted throughout the test procedure.[1]

Twenty-four h following placement of the test animals in their respective vessels, toxicant was randomly added to appropriate containers in 1.0 ml distilled water, giving 25 replications per concentration. Toxicant concentrations were 50, 25, 10, 1.0, 0.1, and zero ppm for shrimp and 50, 25, 20, 15, 10, 1.0, 0.1, and zero ppm for crabs. Observations were at 1, 4, 8, 12, 24, 48, 72, and 96 h for both test species, with additional observations intermittent to the times listed. Symptoms of death were lack of gill movement and particularly no response to touching with a glass rod.

CPTH Extraction and Analysis: Aliquots (100-200 ml) of water were collected from each of two randomly-selected test vessels for each toxicant concentration at 24-h intervals and analyzed for residual CPTH. Each sample was placed in a 500 ml separatory funnel and amended with 1.0 ml 1 N NaOH and 100 ml benzene. The mixture was thoroughly shaken, and the water layer extracted four additional times with fresh 100 ml quantities of benzene. Benzene extracts were pooled, dried by passage through sodium sulfate, and evaporated to dryness.

The effectiveness of the extraction procedure was evaluated by extracting a CPTH-fortified control simultaneously with toxicant-amended water from the test vessels. The control was prepared fresh for each extraction from the stock CPTH solution and in a manner such that the toxicant concentration in the control was identical to that in the water from the test vessels assuming 100% recovery. CPTH recovery in the control extract was used to correct recovery in the treatment extract for fluctuations in the efficiency of the extraction procedure.

[1] In general, testing procedures were performed in accordance with "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphicians," U.S. E.P.A., Corvallis, OR, 1975 (EPA-660/3-75-009).

Following evaporation, both the treatment and control extracts were derivatized using 2, 4-Dinitro-1-fluorobenzene prior to gas chromatographic analysis. Evaporated samples were rinsed 5X with two ml portions of distilled water and placed in 50 ml glass-stoppered erlenmeyer flasks. Borate buffer (0.1 M $Na_2B_4O_7 \cdot 10H_2O$; 10ml) and two ml 1% dinitrofluorobenzene in acetonitrile were added, and the contents mixed. Each flask was then placed in a water bath and heated for one hour at 70 C. A pure CPTH derivative (CPTH plus derivatization reagents but no extraction) and a blank (derivatization reagents only) were also run. After one hour at 70 C, all samples were allowed to cool and the derivatized CPTH was extracted by the addition of 5 or 10 ml benzene. These extracts were quantitated by electron-capture gas chromatography.

A Tracor MT-222 gas chromatograph equipped with a Ni^{63} electron-capture detector was employed. A 0.61 m column packed with 10% DC 200 on 100-120 mesh Chromosorb W HP was used. Column, detector, and inlet temperature were 220, 275, and 235 C respectively. Carrier gas, N_2, flow was 100 ml/min.

CPTH recovery was computed by dividing the peak height observed in the extracted CPTH derivative by that for the pure derivative, multiplying by 100, and correcting for fluctuations in extraction efficiency using the CPTH control.

RESULTS AND DISCUSSION

Shrimp. The mortalities of test shrimp at varying times during the 96-h test period are shown in Table 1. It should be noted that all shrimp were dead after only 8 h exposure to CPTH concentrations of 50 and 25 ppm, while the lower concentrations produced partial mortalities. Preliminary testing using ten replicate white shrimp resulted in total mortality in 30 min at 100 ppm CPTH. These data indicate that at some point between 10 and 25 ppm, a "threshold" concentration is reached above which 100% mortality is observed and below which partial mortalities occur.

In developing the regression line and equation used in calculating the TLM, CPTH concentrations above this "threshold limit" (i. e., 25 and 50 ppm) were not plotted. The resulting regression graph is shown in Figure 1. In this graph, probit kill (BLISS 1935) is plotted against the logarithm of toxicant concentration. The regression equation, $Y = 2.681 + 0.575X$ produces a calculated TLM of 10.8 ppm, a logically realistic value since the actual test concentration of 10 ppm effected an observed mortality of 48% in 96 h. The regression coefficient of 0.999 for the data in Figure 1 indicates an excellent fit of the data to the line.

Shrimp reactions to the higher CPTH concentrations (100, 50, and 25 ppm) were dramatic. Test shrimp jumped about vigorously in efforts to escape, became spastic, shuddered, and died. At lower CPTH concentrations, affected animals exhibited agitation, spastic behavior, loss of equilibrium, and paralysis (on side) prior to

TABLE 1

Percent mortality and statistical significance of penaeid shrimp during 96 hours exposure to varying concentrations of CPTH under standard, static, bioassay conditions.

CPTH Concentration (ppm)	Percent Mortality at Hours Incubation[1]								Statistical Significance[2]
	1	4	8	12	24	48	72	96	
50	80	96	100	100	100	100	100	100	**
25	4	80	100	100	100	100	100	100	**
10	8	16	24	28	28	36	40	48	*
1.0	0	4	4	4	4	8	16	28	NS
0.1	0	0	0	0	0	0	4	12	NS
Control	0	0	0	8	8	8	8	16	--

[1]Twenty-five shrimp were employed at each toxicant concentration and control.

[2]All tests for significance were accomplished by way of a one-way analysis of variance of mortalities at 96 hours. Mortality at each CPTH treatment level was compared to that in the control by F-ratio computation, and the statistical significance of these F-values was ascertained at the 99 and 95 percent level of confidence. Symbols employed are NS, not significant at the 95% level; *, significant at the 95% level but not at the 99% level; and **, significant at the 99% level.

death. Some shrimp remained on their side and unable to right themselves for as long as 72 h prior to death, and others survived throughout the test period on their sides.

Crabs. The mortalities of test crabs at varying times during the 96-h test period are shown in Table 2. Twenty-five replicate crabs were used for all treatments receiving CPTH, while 50 replicates were employed in the control. The data in Table 2 indicate that the crabs began to die after some 8 h of exposure to the higher CPTH concentrations, and that, as was the case for shrimp, an upper "threshold concentration" was reached at 96 h somewhere between 25 and 50 ppm. In the crab study, however, a lower "threshold" was also observed between 1.0 and 10 ppm below which no mortalities were recorded. CPTH concentrations of 25, 20, 15, and 10 ppm reflected partial mortalities and were, therefore, used in the development of the regression equation for the data.

The regression graph for the crab toxicity data is shown in Figure 2. The regression equation, $Y = 21.435 + 6.288X$, produces a calculated TLM of 16.0 ppm. The regression coefficient of 0.979 for this data indicates that the fit is good and that the calculated TLM is valid.

Symptomatically, affected blue crabs exhibited agitation and spastic movements similar to the shrimp, followed by loss of equilibrium, turning upside down, and curled leg appendages. Very little appendage movement was observed. Some exhibited autotomy, casting off chelipeds and pereiopods. Just prior to death, acute paralysis was evident, and movement of mouth parts and gill bailers gradually ceased. In those CPTH concentrations reflecting partial mortalities (10, 15, 20, and 25 ppm), some crabs survived the entire test period on their backs and partially paralyzed.

CPTH Recovery. The percent recovery of CPTH from actual bioassay water during 96 h is shown in Table 3. Considerable difficulty was encountered in the development of a procedure for the quantitative extraction of CPTH from water, seemingly an outgrowth of the compound's affinity for water in preference to other solvents. We have, however, developed a procedure in which we have considerable confidence, especially at CPTH concentrations above 10 ppm.

As indicated in Table 3, CPTH recoveries were lower than generally expected after one h. Even the highest recovery of 86% (shrimp, 50 ppm) is lower than one would expect after so short a period of time. These data indicate that the added CPTH may well be adsorbing to the glass walls of the bioassay vessels in such a manner that a considerable portion of the added toxicant is not available to the water phase of the test container and, hence, to the test organism. The rate of CPTH degradation decreased sharply following

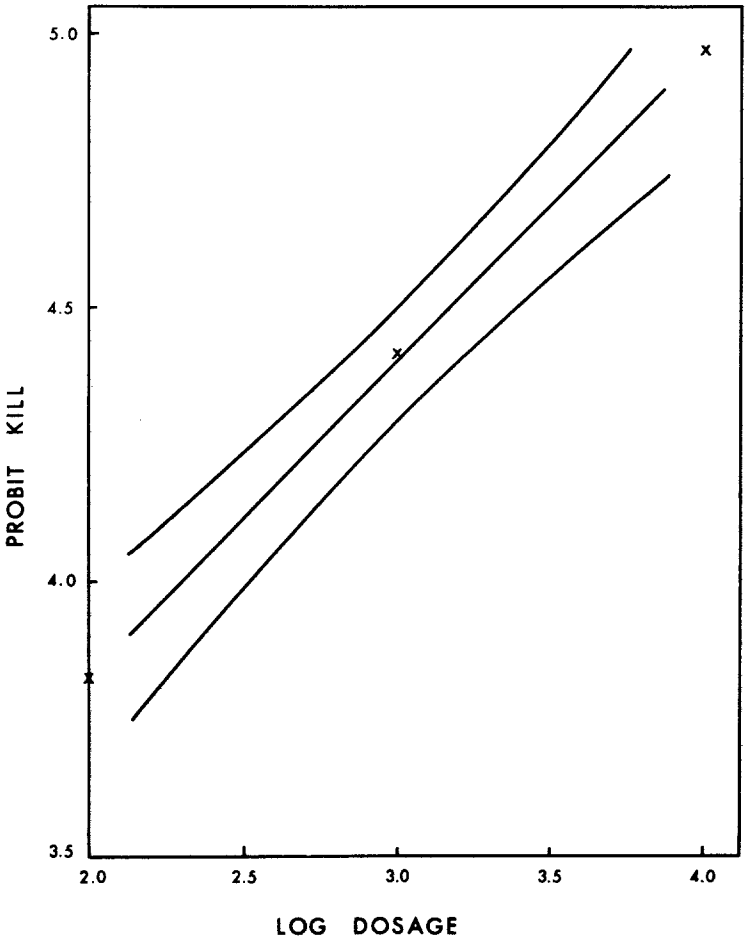

Figure 1. Regression graph of CPTH toxicity to penaeid shrimp during 96 h exposure under standard, static, bioassay conditions.

the one-h extraction, indicating that the CPTH molecule is in all probability not being broken down by chemical or biological mechanisms, giving additional credence to the possibility of starlicide adsorption to the bioassay containers. And, if one accepts adsorption as the mechanism responsible for the diminished CPTH recoveries, it must be realized that such a reduction in CPTH availability to the test organism effects a concomitant reduction in the calculated TLM values. The data generated in this investigation, however, do not demonstrate that such adsorption actually occurs, they merely indicate that such adsorption is certainly a possibility.

TABLE 2

Percent mortality and statistical significance of blue crabs during 96 hours exposure to varying concentrations of CPTH under standard, static, bioassay conditions.

CPTH Concentration (ppm)	Percent Mortality at Hours Incubation[1]								Statistical Significance[2]
	1	4	8	12	24	48	72	96	
50	0	0	20	48	76	100	100	100	**
25	0	0	4	4	24	72	80	88	**
20	0	0	0	4	32	44	72	80	**
15	0	0	0	4	12	28	32	32	**
10	0	0	0	0	0	0	0	12	NS
1.0	0	0	0	0	0	0	0	0	NS
0.1	0	0	0	0	0	0	0	4	NS
Control	0	0	0	0	0	2	2	2	--

[1]Twenty-five crabs were employed at each toxicant concentration and 50 crabs were employed in the control.

[2]All tests for significance were accomplished by way of a one-way analysis of variance of mortalities at 96 hours. Mortality at each CPTH treatment level was compared to that in the control by F-ratio computation, and the statistical significance of these F-values was ascertained at the 99 and 95 percent levels of confidence. Symbols employed are NS, not significant at the 95% level, and **, significant at the 99% level.

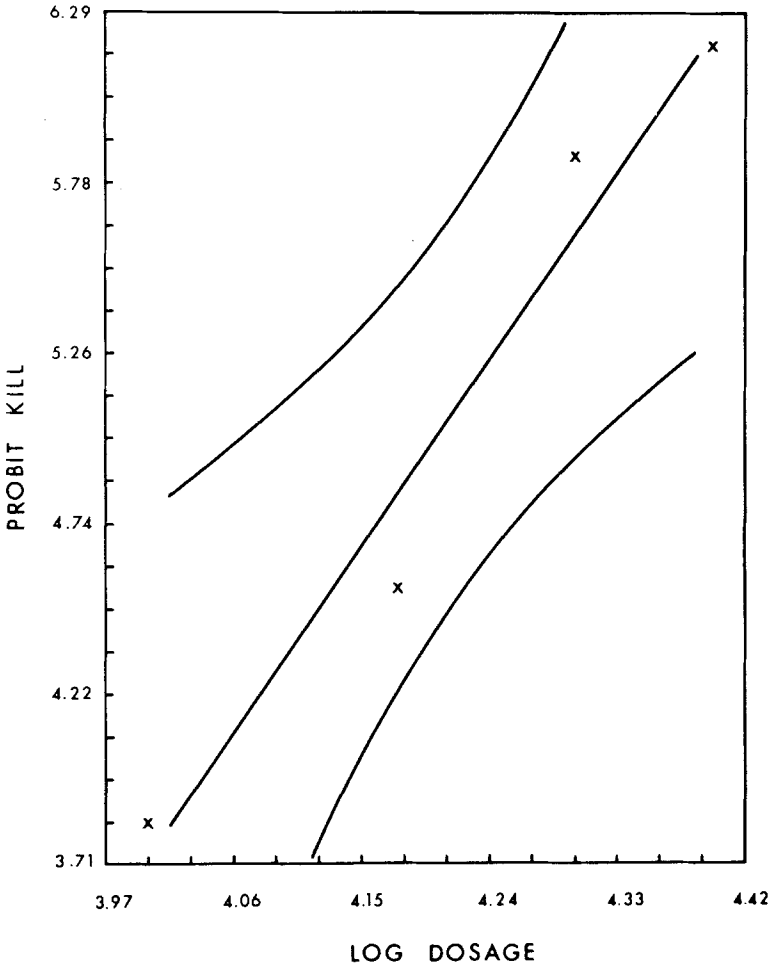

Figure 2. Regression graph of CPTH toxicity to blue crabs during 96 h exposure under standard, static, bioassay conditions.

CONCLUSIONS

The acute toxicity of CPTH to shrimp and crabs was determined in the laboratory under standard, static, bioassay conditions. For shrimp, a TLM of 10.8 ppm was calculated while for crabs a figure of 16.0 ppm was obtained.

These values are relatively high with respect to other pesticide chemicals. However, one must realize that death is a quite serious symptom and that potentially fatal sub-lethal effects are

not discerned in an investigation of this type. Further, we must keep in mind the possibility of toxicant adsorption onto the bioassay vessel and the resulting lowered TLM values. The assessment of these factors, however, was not within the scope of this investigation.

TABLE 3

Percent recovery[1] of CPTH from shrimp and crab bioassay water during 96 hours under standard, static, bioassay conditions.

Time (Hours)	CPTH Concentration (ppm) 50	25	10	1.0	0.1
			Shrimp		
1	86±0	66±1	66±3	6±0	--
24	75±3	72±3	50±6	5±0	4±0
48	75±1	69±1	43±4	4±0	7±2
72	63±1	52±10	48±1	4±0	12±0
96	66±0	55±2	43±1	4±0	9±2
			Crabs		
1	60±0	57±0	49±3	17±0	17±1
24	54±0	57±4	63±4	6±0	12±1
48	51±0	57±0	65±1	6±0	11±0
72	56±2	55±0	50±0	5±1	11±0
96	55±2	57±0	42±9	5±0	10±0

[1]All values have been corrected for deviations of control recoveries from 100%, which ranged from 77.9 to 104.0.

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REFERENCES

BLISS, C. I.: An. Appl. Biol. 22, 134 (1935).

DeCINO, T. J., D. J. CUNNINGHAM, and W. E. SCHAFER, JR.: J. Wildl. Manage. 30 (2), 249 (1966).