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# PRELIMINARY EVALUATION OF THE ACUTE TOXICITY OF DESENSITIZED PRIMER COMPOUNDS AND PRIMER WASTE EFFLUENTS TO REPRESENTATIVE AQUATIC ORGANISMS

BY

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The acute toxicity of five desensitized primer compounds and primer manufacturing waste effluents to three freshwater species, Daphnia magna (water flea:crustacean), Lepomis macrochirus (bluegill:fish) and Pimephales promelas (fathead minnow:fish) was determined in static bioassays. The primers tested were trinitroresorcinol (TNR =styphnic acid, lead styphnate (PbTNR), tetracene, pentaerythritol tetranitrate (PETN) and FA 956 priming mixture. The waste effluents were those resulting from the production of TNR, PbTNR,

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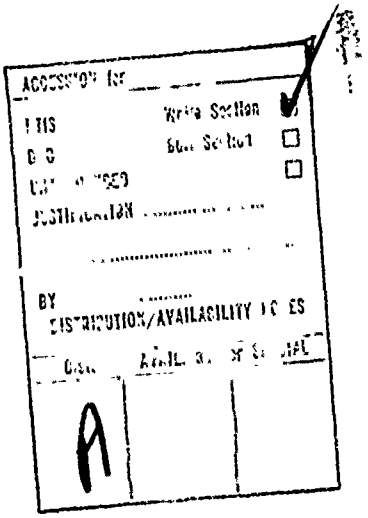
20. ABSTRACT (Continued)

tetracene and FA 956. In addition, a reagent blank which contained no primer materials but was desensitized by the procedure used for TNR, was also tested. To determine the effect of the high pH, resulting from the desensitization process, of test materials, bioassays were performed with Daphnia magna and Pimephales promelas exposed to neutralized versus unneutralized materials.

Results indicated that, except for tetracene, the acute toxicity of the primers and waste effluents tested was due primarily to the high pH resulting from desensitization.

Daphnia magna were more sensitive than were fish to the neutralized primer materials (except tetracene) tested. Tetracene and FA 956 were similar in toxicity and were significantly more toxic to Daphnia magna than were the other primers.

Both fish species were equally susceptible to the desensitized test materials.



## SUMMARY

1. For all primer materials studied (except tetracene) the acute toxicity to aquatic organisms of both the desensitized primer compound and wastewaters resulting from primer manufacturing processes was largely due to the high pH (>12.0) resulting from the desensitization process.

2. The acute toxicity of neutralized samples of primer materials indicated that Daphnia magna were significantly more susceptable to all samples (except tetracene) then were bluegills and fathead minnows.

3. Of the five desensitized primer materials tested, tetracene and primer mixture FA 956 are clearly the most toxic to daphnids, with 48-hour LC50 values for these two materials being several orders of magnitude lower than those observed for lead styphnate, PETN and TNR. The toxicity of all desensitized materials tested to both fish species was much less variable and 96-hour LC50 values ranged from 1.9-13.4% v/v (66-1085 mg/l, total solids), with no significant difference observed between fish species.

4. The data suggest that additional information on the acute toxicity of these materials to other aquatic forms, and on the effects of continuous exposure to these materials on aquatic

organisms are essential to an evaluation of the potential hazard associated with the introduction of these materials into aquatic ecosystems. Based on the relative acute toxicity of the materials studied, emphasis for additional research information should be on tetracene and primer mixture FA 956.

## INTRODUCTION

Studies were conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts, to provide the U.S. Army Medical Research and Development Command with information to evaluate the relative toxicity to aquatic organisms of desensitized primer compounds and primer manufacturing waste effluents and to establish research priorities for these materials. Bioassays were conducted in which an aquatic invertebrate, Daphnia magna (water flea), and two species of freshwater fish Lepomis macrochirus (bluegill), and Pimephales promelas (fathead minnow), were exposed to each of these materials to assess acute toxicity under standard, static test conditions.

## MATERIALS AND METHODS

The methodology for conducting these acute toxicity tests closely followed the recommended bioassay procedures as described in the Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians (EPA, 1975). These procedures reflect the most recent state-of-the-art methodology for acute toxicity studies with aquatic organisms.

The materials evaluated during bioassays were trinitroresorcinol (TNR; = styphnic acid), lead styphnate (PbTNR), tetracene (= tetrazene), pentaerythritol tetranitrate (PETN), and FA 956 priming mixture. The FA 956 mixture is composed of: 37% PbTNR, 4% tetracene, 5% PETN, 32% barium nitrate, 15% antimony sulfide and 7% aluminum (Small and Rosenblatt, 1974).

Each of these primer components was desensitized at the Frankford Arsenal, Philadelphia, Pa., prior to shipment to Bionomics, for use in toxicity tests. Desensitization involved boiling (by steam injection) the primer material in water under chemically reducing conditions for a minimum of 4 hours in batch process. To prepare desensitized TNR, PbTNR, PETN and FA 956 for use in these bioassays, each compound was boiled separately in a solution of water, sodium hydroxide and atomized aluminum. Tetracene was boiled with water alone. In addition to these desensitized primer compounds, the acute toxicity of

samples of the wastewater discharged from the production of TNR, PbTNR, tetracene, and primer mixture FA 956 was also evaluated. These desensitized wastewaters were not synthesized but were collected from an actual treatment facility at the Lake City Army Ammunition Plant, Independence, Missouri. The primers and waste effluents were all tested as 100% active. Finally, the acute toxicity of a desensitization process reagent blank was also determined. This blank contained no primer material but was desensitized by the procedure used for TNR. The pH and total solids (%, weight/volume basis) of all samples except manufacturing wastewaters are summarized in Table 1. Duplicate samples of each primer compound were analyzed for total solids as outlined in the Methods for Chemical Analysis of Water and Wastes (EPA, 1974).

Results for all tests were expressed as the median lethal concentration (LC50). The criterion utilized in bioassays was death. The LC50 values and their 95% confidence intervals were calculated by converting the test concentrations and the corresponding observed percentage mortality to logs and probits, respectively. These values were then utilized in a least squares regression analysis, and the LC50 values and their 95% confidence limits were estimated from the calculated regression equations.

The bluegill (Lepomis macrochirus) used in these tests were acquired from a commercial fish hatchery in Nebraska and had a mean wet weight and standard length of 1.1 g and 39 mm, respectively. The fathead minnow (Pimephales promelas) were obtained from a fish farmer in Arkansas, and had a mean wet weight of 1.0 g and a mean standard length of 43 mm. The water flea (Daphnia magna) utilized in these bioassays were acquired from Bionomics' laboratory cultures and were less than 24 hours old at the beginning of a bioassay.

Prior to use in bioassays, all test fish were held in 1700-l concrete raceways which were coated with an epoxy resin paint to prevent leaching of materials into the water. Flow of well water (having a temperature of 21 ± 1.0°C, hardness of 35 mg/l as $CaCO_3$, pH of 7.1, and dissolved oxygen concentration of >60% of saturation) into these raceways was at least 4 l/minute, which provided an adequate rate of turnover for holding these species. The fish were held in the laboratory hatchery facilities for at least thirty days prior to testing. During this period, cumulative mortality was <2%; no mortality was observed during the 48 hours immediately prior to testing, and these fish were judged to be in excellent condition. Fish of each species were from the same year class, and the standard length of the longest fish was no more than two times that of the shortest fish. Prior to exposure, the fish were acclimated

over a 48-hour period to test conditions of temperature and water quality and were not fed during this period.

Due to the extremely high alkalinity of each desensitized primer compound, primer wastewater (excluding tetracene and tetracene wastewater) and the blank, it was deemed necessary to conduct preliminary bioassays utilizing fathead minnows exposed to the unneutralized compounds to determine if toxicity was a function of pH or of the test compound. Similarly, Daphnia were exposed to unneutralized and neutralized samples of the reagent blank. The relatively high degree of sensitivity exhibited by fathead minnows to all unneutralized samples, including the desensitization reagent blank, and by Daphnia to the reagent blank, indicated that at least part of this toxicity was due to high pH. Because of the relatively high toxicity due to pH, all of the compounds except tetracene and tetracene wastewater were subsequently assayed after neutralization. The pH of each test material was lowered to 7.0, except for tetracene, which was received at a pH of 6.1 and tetracene wastewater (pH 8.9). Neutralization was achieved through addition of concentrated sulfuric acid, dropwise, to the primer wastewaters.

The toxicity of two series of concentrations was tested within each bioassay, a series of range-finding concentrations, and a series of definitive concentrations. The preliminary test was

conducted to determine an approximate range of concentrations for evaluating the dose-response relationship. The definitive test, consisting of at least five concentrations, evaluated the dose-response relationship to a degree allowing the LC50 to be calculated from the data with optimum accuracy. The number of concentrations tested, depended, in part, on the volume of test materials available. A control, which consisted of the same dilution water, conditions, and organisms, was maintained for each species tested. The vessels were not aerated throughout the test period. Fish were introduced into each test vessel within thirty minutes after the compound was added.

Due to the extremely limited available volume of both the primer compounds and the primer wastewaters, it was necessary to conduct the assays in smaller volumes of water than those normally recommended in the bioassay procedures. Normally, exposures of 10 fish at each of the 5 or more test concentrations are conducted coincidentally. In these studies, however, fish bioassays were performed in replicate 600 ml beakers containing 250 ml of solution at a concentration of 100% primer material or wastewater. Water temperature was maintained at 21 ± 1.0°C for all fish bioassays. In order to maintain an acceptable dissolved oxygen concentration, only 2-3 fish were exposed at each concentration. Upon observation of 100% mortality, the solution was then diluted to a successively lower concentration

and its toxicity was tested. This procedure enabled maximum usage of the available volumes of materials to be tested and effectively increased the volume of the test solution from a minimum of 250 ml to approximately 3 l. The potential for error in estimates of toxicity as a result of this sequential dilution testing procedure is considered in the "Results and Discussion" section which follows. The water flea bioassays were conducted in 250 ml beakers containing 50 ml of solution. This 50 ml volume was held constant throughout the testing of the primer compounds and waste effluents. Five Daphnia (<24 hours old) were introduced into each of 3 replicates per concentration tested. The reduced number of Daphnia tested per replicate was a result of the smaller volumes of test solution used due to the limited volumes of test materials which were available. Water temperature in the Daphnia bioassays was maintained at 22 ± 1.0°C. Prior to introducing primer materials into test containers, samples were mixed in an attempt to resuspend and homogeneously distribute solids which were present.

## RESULTS AND DISCUSSION

The results of the preliminary bioassays with fathead minnows and the unneutralized samples of the desensitization blank and desensitized primer samples clearly indicate a significant toxic effect due to the strongly alkaline pH resulting from the desensitization process (Table 2 and 3). The pH's of test materials as received were strongly alkaline, ranging from 12.1 to 14.0, except for the tetracene and its wastewater. Furthermore, based on information available in the scientific literature, at the aqueous concentration tested, the pH's alone of the diluted materials were sufficiently high (9.5 to 11.0; Table 3) to have produced the observed mortalities. This conclusion was further supported by the observed lower toxicity of the tetracene and tetracene wastewater samples which had pH's of 6.1 and 8.9, respectively, after desensitizatic

EPA Water Quality Criteria (1973) recommends that for the protection of freshwater biota, the pH of natural freshwaters should not exceed 8.5 to 9.5. However, pH's of 9.0 and above are likely to produce mortality in several species of freshwater fish. In addition, these higher pH's are favorable for the formation of undissociated ammonium hydroxide ($NH_4OH$) and un-ionized ammonia ($NH_3$) in amounts which may be toxic. Daye and Garside (1975) reported that there are relatively few data

regarding the upper and lower lethal limits of pH for freshwater fish. However, the data which are available, indicate that the upper lethal limits are from 8.7 to 11.1. Bluegill (Lepomis macrochirus) have been shown to have an upper lethal limit of pH 10.5. Daye and Garside (1975) empirically determined that the upper lethal limit for brook trout (Salvelinus fontinalis) ranged from 9.5 to 10.0 with a mean of 9.8. Furthermore, temperature differences of 10°C (tests performed at 10 and 20°C) did not affect this limit.

Freshwater invertebrates are also sensitive to waters with a strongly alkaline pH. Thornton and Wilhm (1975) have demonstrated that survival of eggs, larval stages (instars) and adult midges (Chironomus attenuatus) was significantly reduced by exposure to water with a pH of 8.2 as compared with pH's of 7.2 and 6.2.

The results of the bioassays utilizing daphnids and samples of both neutralized and unneutralized desensitization reagent blank, clearly document the toxicity associated with the high pH of the blank (Table 4). As reported, the toxicity of the unneutralized reagent blank to daphnids is at least 200X greater than that of the neutralized blank. The toxicity data presented for daphnids and neutralized primer samples (except for tetracene and tetracene waste effluent) are

representative of the toxicity of the chemicals associated with the primer, per se, and not of that related to the desensitization process. These data (Table 4 and 5) indicate that of the five primer materials tested, tetracene and primer mixture FA 956 are clearly the most toxic to Daphnia. The calculated 48-hour LC50, based on nominal concentrations, for unneutralized tetracene and daphnids was 0.004% v/v (40 ppm), and that for primer mixture FA 956 was 0.0029% v/v (29 ppm). The acute toxicity (48-hour LC50) of unneutralized lead styphnate, PETN and TNR to daphnids is clearly several orders of magnitude less than that determined for tetracene and primer mixture FA 956, and ranges from 0.16% v/v for lead styphnate to 0.85% v/v for PETN.

Primer wastewaters were significantly less toxic to Daphnia than were the primer components. The most toxic wastewater was that resulting from the TNR manufacturing process; a 48-hour LC50 of 1.6% v/v was calculated. Primer mixture FA 956 wastewater appeared to be the least toxic of those tested with an estimated 48-hour LC50 of 13.2%.

The acute toxicity (96-hour LC50) of all samples to bluegills and fathead minnow is summarized in Tables 6 and 7. The 96-hour LC50 reported here for the neutralized desensitization blank and fathead minnows, when compared to that reported

for the unneutralized desensitization blank (Table 2) again is an indication of the significance of the toxicity due to pH of the unneutralized desensitization blank to aquatic organisms. Clearly, the toxicity of the unneutralized blank is approximately 2 orders of magnitude greater than that of the neutralized blank.

The acute toxicity of all samples tested was similar for both fishes, with no significant differences in sensitivity between species evident. A comparison of the susceptibility of daphnids and fishes to the primer materials clearly indicates that the Daphnia are significantly more sensitive than fishes to all primer materials during exposure. The most obvious example of this susceptibility is evident from a comparison of the 48-hour LC50 of the desensitized tetracene primer to Daphnia (0.004% v/v) with the 96-hour LC50 of the same material to bluegills (3.5% v/v) or fathead minnows (1.9% v/v). A similar observation can be made for primer mixture FA 956.

The total solids content of test samples at the concentrations equivalent to the 48-hour LC50 for water flea and to the 96-hour LC50 for fish was calculated based on the measured total solids in the test samples as received and on the subsequent dilution utilized to produce these test concentrations (Table 8). Relating the toxic concentrations of primer compounds, wastewaters and the reagent blank to their corresponding total solids content may

provide a convenient method of assessing potential hazards of these chemicals to aquatic organisms.

As mentioned in the Materials and Methods section, the limited amounts of primer compounds and wastewaters necessitated sequential dilution testing methods which vary from standard bioassay procedures. However, the result of these tests indicate a typical dose-response relationship thus supporting the stated conclusions. Furthermore, since this was a screening program, these data are adequate for the determination of relative toxicity of test materials.

An evaluation of the acute toxicity of primer waste effluents to fishes would suggest that reasonable dilution of the neutralized wastewater in the receiving water would preclude short-term acute effects on fish populations. However, these data clearly indicate that neutralization of primer wastewaters (except tetracene wastewater) is necessary to significantly reduce toxicity to aquatic organisms.

The preliminary data have suggested that acute toxicity data on a variety of aquatic organisms is required to evaluate environmental hazard since the susceptibility of aquatic organisms to primer materials varies greatly. In view of the relatively high acute toxicity of some materials (e.g.,primary mixture FA 956 and tetracene) to daphnids,

information on the effects of long-term or continuous exposure (as might be the case in a receiving water) would be desirable in completing a realistic hazard evaluation. If significant differences in toxicity to daphnids are observed during long-term or continuous exposure, similar studies with fishes may be required to adequately assess hazards.

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Table 1 - Desensitized primer compounds, wastewaters and a reagent blank, included in a research program to evaluate the acute toxicity of these compounds to bluegill (Lepomis macrochirus), fathead minnow (Pimephales promelas), and water flea (Daphnia magna).

| Compound | pH | Total solids (%, w/v)[a] |
|---|---|---|
| reagent blank | 13.2 | 0.81 (0.06)[b] |
| PbTNR | 12.6 | 0.44 (0.01) |
| PbTNR wastewater (Bldg. 85) | 12.1 | 0.17 (0.00) |
| PETN | 14.0 | 3.43 (0.25) |
| Tetracene | 6.1 | 0.35 (0.11) |
| Tetracene wastewater (Bldg. 85) | 8.9 | 0.01 (0.00) |
| TNR | 13.1 | 0.77 (0.00) |
| TNR wastewater (Bldg. 83) | 12.4 | 1.36 (0.04) |
| FA 956 | 12.6 | 0.69 (0.00) |
| FA 956 wastewater (Bldg. 90C) | 12.3 | 0.07 (0.00) |

[a]Results are the mean of duplicate analyses

[b]Standard deviation

Table 2 - Preliminary estimated acute toxicity of unneutralized desensitized primer compounds, wastewaters and a reagent blank, to fathead minnow[a] (Pimephales promelas). These data are based on results of preliminary bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

| Compound | LC50 (%, v/v) | | | No discernible effect level (%, v/v) |
|---|---|---|---|---|
| | 24 hour | 48 hour | 96 hour | |
| reagent blank | >0.10<0.32 | >0.10<0.32 | >0.10<0.32 | 0.10 |
| PbTNR | >1.00 | >0.32<1.00 | >0.32<1.00 | 0.32 |
| PbTNR wastewater | >1.00 | >1.00 | >0.32<1.00 | 0.32 |
| PETN | >0.032<0.100 | >0.032<0.100 | >0.032<0.10 | 0.032 |
| Tetracene | 9.4 (2.7-32)[b] | >1.0<3.2 | >1.0<3.2 | 1.0 |
| Tetracene wastewater | >100 | >100 | >100 | 100 |
| TNR | 0.30 (0.09-1.02) | 0.30 (0.09-1.02) | 0.30 (0.09-1.02) | 0.10 |
| TNR wastewater | >1.00 | >0.32<1.00 | >0.32<1.00 | 0.32 |
| FA 956 | >0.32<1.00 | >0.32<1.00 | >0.32<1.00 | 0.32 |
| FA 956 wastewater | 2.96 (0.86-10.20) | >1.00<3.20 | >1.00<3.20 | 1.00 |

[a]Bioassays conducted at 12 ± 1.0°C; mean weight of fathead minnow, 1.0 g

[b]95% confidence interval

Table 3 - Concentrations tested and corresponding observed percentage mortalities for fathead minnow (Pimephales promelas) exposed to unneutralized desensitized primer compounds, wastewaters and a reagent blank, for 24, 48, and 96 hours. These data are based on the results of preliminary bioassays conducted at the Acute Toxicity Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

| Compound | Concentration (%, v/v) | pH | N[a] | % mortality observed | | |
|---|---|---|---|---|---|---|
| | | | | 24 hour | 48 hour | 96 hour |
| reagent blank | 0.32 | 10.9 | 2 | 100 | 100 | 100 |
| | 0.10 | 10.1 | 2 | 0 | 0 | 0 |
| | Control | | 2 | 0 | 0 | 0 |
| PbTNR | 1.0 | 9.9 | 2 | 50 | 100 | 100 |
| | 0.32 | 10.0 | 2 | 0 | 0 | 0 |
| | 0.10 | 8.0 | 2 | 0 | 0 | 0 |
| | Control | | 2 | 0 | 0 | 0 |
| PbTNR wastewater | 1.0 | 9.5 | 2 | 50 | 50 | 100 |
| | 0.32 | 8.9 | 2 | 0 | 0 | 0 |
| | 0.10 | 7.5 | 2 | 0 | 0 | 0 |
| | Control | | 2 | 0 | 0 | 0 |
| PETN | 0.100 | 11.0 | 2 | 100 | 100 | 100 |
| | 0.032 | 10.2 | 2 | 0 | 0 | 0 |
| | Control | | 2 | 0 | 0 | 0 |

Table 3 - continued

| Compound | Concentration (%, v/v) | pH | N[a] | % mortality observed | | |
|---|---|---|---|---|---|---|
| | | | | 24 hour | 48 hour | 96 hour |
| Tetracene | 32 | 5.9 | 2 | 100 | 100 | 100 |
| | 10 | 5.9 | 2 | 50 | 100 | 100 |
| | 3.2 | 6.1 | 2 | 0 | 100 | 100 |
| | 1.0 | 6.5 | 2 | 0 | 0 | 0 |
| | Control | | 2 | 0 | 0 | 0 |
| Tetracene wastewater | 100 | 8.9 | 2 | 0 | 0 | 0 |
| | 10 | - | 2 | 0 | 0 | 0 |
| | 1.0 | 7.2 | 2 | 0 | 0 | 0 |
| | 0.32 | 8.2 | 2 | 0 | 0 | 0 |
| | Control | | 2 | 0 | 0 | 0 |
| TNR | 1.0 | 10.1 | 2 | 100 | 100 | 100 |
| | 0.32 | 9.9 | 2 | 50 | 50 | 50 |
| | 0.10 | 9.6 | 2 | 0 | 0 | 0 |
| | Control | | 2 | 0 | 0 | 0 |
| TNR wastewater | 1.0 | 10.7 | 2 | 50 | 100 | 100 |
| | 0.32 | 9.7 | 2 | 0 | 0 | 0 |
| | 0.10 | 9.1 | 2 | 0 | 0 | 0 |
| | Control | | 2 | 0 | 0 | 0 |

Table 3 - continued

| Compound | Concentration (%, v/v) | pH | N[a] | % mortality observed | | |
|---|---|---|---|---|---|---|
| | | | | 24 hour | 48 hour | 96 hour |
| FA 956 | 1.0 | 10.0 | 2 | 100 | 100 | 100 |
| | 0.32 | 9.5 | 2 | 0 | 0 | 0 |
| | 0.10 | 8.8 | 2 | 0 | 0 | 0 |
| | Control | | 2 | 0 | 0 | 0 |
| FA 956 wastewater | 100 | 12.3 | 2 | 100 | 100 | 100 |
| | 10.0 | 10.8 | 2 | 100 | 100 | 100 |
| | 3.2 | 10.3 | 2 | 50 | 100 | 100 |
| | 1.0 | 9.5 | 2 | 0 | 0 | 0 |
| | 0.32 | 9.0 | 2 | 0 | 0 | 0 |
| | Control | | 2 | 0 | 0 | 0 |

[a]N=number of fish tested/concentration.

Table 4 - Acute toxicity of desensitized primer compounds, wastewaters and a reagent blank, to the water flea[a] (Daphnia magna). The data are based on the results of acute static bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

| Compound | LC50 (%,v/v) 24 hour | LC50 (%,v/v) 48 hour | No discernible effect level (%, v/v) |
|---|---|---|---|
| reagent blank (unneutralized) | >0.0100 | 0.0056 (0.0044-0.0073) | 0.0024 |
| reagent blank (neutralized) | >1.0<10 | >1.0<10 | 1.0 |
| PbTNR (neutralized) | >0.560 | 0.160 (0.100-0.230) | 0.075 |
| PbTNR wastewater (neutralized) | 8.43 (5.77-12.50)[b] | 3.00 (0.58-1.24) | 1.00 |
| PETN (neutralized) | 0.97 (0.75-1.26) | 0.85 (0.58-1.24) | 0.56 |
| Tetracene (unneutralized) | 0.0056 (0.0042-0.0075) | 0.0040 (0.0034-0.0048) | 0.0028 |
| Tetracene wastewater (unneutralized) | 14.7 (2.44-89.0) | 4.52 (2.27-9.02) | 0.75 |
| TNR (neutralized) | >1.0<10 | 0.68 (0.37-1.24) | 0.18 |
| TNR wastewater (neutralized) | >1.8<2.4 | 1.6 (1.3-2.0) | 1.0 |
| FA 956 (neutralized) | 0.0037 (0.0030-0.0044) | 0.0029 (0.0024-0.0036) | 0.0018 |
| FA 956 wastewater (neutralized) | 24.2 (14.0-41.7) | 13.2 (10.1-17.2) | 5.6 |

[a]Bioassays conducted at 22 ± 1.0°C; water flea less than 24 hours old prior to the initiation of testing

[b]95% confidence interval

Table 5 - Concentrations tested and corresponding observed percentage mortality for Daphnia magna exposed to primer compounds, wastewaters and a reagent blank, for 24 and 48 hours. Each mortality value represents the mean of 3 replicates.

| Compound | Concentration (%, v/v) | % mortality observed 24 hour | 48 hour |
|---|---|---|---|
| reagent blank (unneutralized) | 0.0100 | 7 | 100 |
| | 0.0075 | 13 | 33 |
| | 0.0056 | 0 | 7 |
| | 0.0042 | 7 | 13 |
| | 0.0032 | 7 | 13 |
| | 0.0024 | 0 | 0 |
| | Control | 0 | 0 |
| reagent blank (neutralized) | 100 | 100 | 100 |
| | 10 | 100 | 100 |
| | 1.0 | 0 | 0 |
| | Control | 0 | 0 |
| PbTNR (neutralized) | 0.560 | 0 | 100 |
| | 0.420 | 53 | 100 |
| | 0.180 | 0 | 53 |
| | 0.100 | 0 | 20 |
| | 0.075 | 0 | 0 |
| | Control | 0 | 0 |
| PbTNR wastewater (neutralized) | 18.0 | 100 | 100 |
| | 10.0 | 67 | 100 |
| | 5.60 | 13 | 87 |
| | 3.20 | 0 | 67 |
| | 1.00 | 0 | 0 |
| | Control | 0 | 0 |
| PETN (neutralized) | 1.40 | 7 | 100 |
| | 1.20 | 7 | 100 |
| | 1.00 | 13 | 43 |
| | 0.75 | 0 | 20 |
| | 0.56 | 0 | 0 |
| | Control | 0 | 0 |
| Tetracene (unneutralized) | 0.0075 | 100 | 100 |
| | 0.0056 | 33 | 93 |
| | 0.0042 | 0 | 73 |
| | 0.0032 | 0 | 33 |
| | 0.0028 | 0 | 0 |
| | Control | 0 | 0 |

Table 5 - Continued

| Compound | Concentration (%, v/v) | % mortality observed 24 hour | 48 hour |
|---|---|---|---|
| Tetracene wastewater (unneutralized) | 12 | 63 | 100 |
| | 10 | 20 | 47 |
| | 5.60 | 7 | 13 |
| | 1.00 | 0 | 7 |
| | 0.75 | 0 | 0 |
| | Control | 0 | 0 |
| TNR (neutralized) | 10 | 100 | 100 |
| | 1.0 | 0 | 60 |
| | 0.75 | 0 | 53 |
| | 0.56 | 13 | 53 |
| | 0.32 | 0 | 60 |
| | 0.18 | 0 | 0 |
| | Control | 0 | 0 |
| TNR wastewater (neutralized) | 3.2 | 100 | 100 |
| | 2.4 | 100 | 100 |
| | 1.8 | 0 | 33 |
| | 1.4 | 13 | 13 |
| | 1.0 | 0 | 0 |
| | Control | 0 | 0 |
| FA 956 (neutralized) | 0.0056 | 100 | 100 |
| | 0.0042 | 47 | 60 |
| | 0.0032 | 27 | 80 |
| | 0.0024 | 0 | 60 |
| | 0.0018 | 0 | 0 |
| | Control | 0 | 0 |
| FA 956 wastewater (neutralized) | 24 | 47 | 100 |
| | 21 | 7 | 53 |
| | 18 | 0 | 47 |
| | 14 | 13 | 53 |
| | 10 | 0 | 60 |
| | 5.6 | 0 | 0 |
| | Control | 0 | 0 |

Table 6 - Acute toxicity of neutralized[a] desensitized primer compounds, wastewaters and a reagent blank, to bluegill[b] (Lepomis macrochirus) and fathead minnow[c] (Pimephales promelas). These data are based on results of bioassays conducted at the Aquati Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

| Compound/Species | LC50 (%, v/v) 24 hour | 48 hour | 96 hour | No discernible effect level (%, v/v) |
|---|---|---|---|---|
| reagent blank/ bluegill | >10<14 | >10<14 | >10<14 | 10 |
| reagent blank/ fathead minnow | >18<32 | >14<18 | 13.4 (9.9-17.9)[d] | 10 |
| PbTNR/bluegill | 13.5 (9.8-18.6) | 13.2 (9.6-18.1) | 13.2 (9.6-18.1) | 10 |
| PbTNR/ fathead minnow | 18.1 (13.6-24.2) | >14<18 | 13.2 (9.6-18.1) | 10 |
| PbTNR wastewater/ bluegill | 8.9 (5.4-14.9) | 8.9 (5.4-14.9) | 8.9 (5.4-14.9) | 5.6 |
| PbTNR wastewater/ fathead minnow | >10<14 | >10<14 | 10 (7.2-14.1) | 7.5 |
| PETN/bluegill | >6.5<10 | >6.5<10 | >6.5<10 | 6.5 |
| PETN/ fathead minnow | >3.2<6.5 | >3.2<6.5 | 2.7 (0.95-7.6) | 1.0 |
| Tetracene/ bluegill | >4.2<5.6 | 3.6 (2.1-6.5) | 3.5 (2.5-4.8) | 2.1 |
| Tetracene/ fathead minnow | 3.1 (2.3-4.2) | 3.1 (2.3-4.2) | 1.9 (1.0-3.9) | 1.0 |
| Tetracene wastewater/ bluegill | >100 | >100 | >100 | 100 |

Table 6 - Continued

| Compound/Species | LC50 (%, v/v) 24 hour | 48 hour | 96 hour | No discernible effect level (%, v/v) |
|---|---|---|---|---|
| Tetracene wastewater/ fathead minnow | >100 | >100 | >100 | 100 |
| TNR/bluegill | >2.4<3.2 | >2.4<3.2 | >2.4<3.2 | 2.4 |
| TNR/fathead minnow | >7.5<10 | >7.5<10 | >7.5<10 | 7.5 |
| TNR wastewater/ bluegill | 7.1 (4.8-10.5) | 7.1 (4.8-10.5) | 7.1 (4.8-10.5) | 4.9 |
| TNR wastewater/ fathead minnow | >4.9<7.5 | 5.1 (3.5-7.4) | 5.1 (3.5-7.4) | 3.7 |
| FA 956/bluegill | 9.9 (7.1-13.9) | 9.9 (7.1-13.9) | 7.4 (5.5-10.2) | 5.6 |
| FA 956/ fathead minnow | 7.4 (5.5-10.1) | 5.6 (4.1-7.6) | 5.4 (4.0-7.4) | 4.2 |
| FA 956 wastewater/ bluegill | >75<87 | >75<87 | 70.5 (55.0-90.3) | 56 |
| FA 956 wastewater/ fathead minnow | >75<100 | >65<75 | >65<75 | 65 |

[a]All samples except tetracene and tetracene wastewater were neutralized to pH 7.0 with sulfuric acid.

[b]Bioassays conducted at 21 ± 1.0°C; mean weight of bluegill, 1.1 g.

[c]Bioassays conducted at 21 ± 1.0°C; mean weight of fathead minnow, 1.0 g

[d]95% confidence interval.

Table 7 - Concentrations tested and corresponding observed percentage mortalities for bluegill (Lepomis macrochirus) and fathead minnow (Pimephales promelas) exposed to neutralized desensitized primer compounds, wastewaters and a reagent blank, for 24, 48, and 96 hours.

| Compound/Species | Concentration (%, v/v) | N[a] | % mortality observed | | |
|---|---|---|---|---|---|
| | | | 24 hour | 48 hour | 96 hour |
| reagent blank/bluegill | 32 | 2 | 100 | 100 | 100 |
| | 18 | 2 | 100 | 100 | 100 |
| | 14 | 2 | 100 | 100 | 100 |
| | 10 | 2 | 0 | 0 | 0 |
| | 1 | 2 | 0 | 0 | 0 |
| | Control | 2 | 0 | 0 | 0 |
| reagent blank/ fathead minnow | 32 | 2 | 100 | 100 | 100 |
| | 18 | 2 | 0 | 100 | 100 |
| | 14 | 2 | 0 | 0 | 50 |
| | 10 | 2 | 0 | 0 | 50 |
| | 1 | 2 | 0 | 0 | 0 |
| | Control | 2 | 0 | 0 | 0 |
| PbTNR/bluegill | 24 | 2 | 100 | 100 | 100 |
| | 18 | 2 | 100 | 100 | 100 |
| | 14 | 2 | 33 | 67 | 67 |
| | 10 | 2 | 0 | 0 | 0 |
| | 7.5 | 2 | 0 | 0 | 0 |
| | Control | 2 | 0 | 0 | 0 |
| PbTNR/fathead minnow | 32 | 2 | 100 | 100 | 100 |
| | 24 | 2 | 100 | 100 | 100 |
| | 18 | 2 | 33 | 100 | 100 |
| | 14 | 2 | 0 | 0 | 67 |
| | 10 | 2 | 0 | 0 | 0 |
| | Control | 2 | 0 | 0 | 0 |
| PbTNR wastewater/ bluegill | 14 | 3 | 100 | 100 | 100 |
| | 10 | 3 | 50 | 50 | 50 |
| | 7.5 | 3 | 33 | 33 | 33 |
| | 5.6 | 3 | 0 | 0 | 0 |
| | 4.2 | 3 | 0 | 0 | 0 |
| | Control | 3 | 0 | 0 | 0 |
| PbTNR wastewater/ fathead minnow | 18 | 2 | 100 | 100 | 100 |
| | 14 | 3 | 100 | 100 | 100 |
| | 10 | 3 | 0 | 0 | 33 |
| | 7.5 | 3 | 0 | 0 | 0 |
| | 5.6 | 3 | 0 | 0 | 0 |
| | Control | 3 | 0 | 0 | 0 |

Table 7 - Continued

| Compound/Species | Concentration (%, v/v) | N[a] | % mortality observed | | |
|---|---|---|---|---|---|
| | | | 24 hour | 48 hour | 96 hour |
| PETN/bluegill | 10 | 2 | 100 | 100 | 100 |
| | 6.5 | 3 | 0 | 0 | 0 |
| | 4.2 | 3 | 0 | 0 | 0 |
| | 2.8 | 3 | 0 | 0 | 0 |
| | 1.8 | 3 | 0 | 0 | 0 |
| | Control | 3 | 0 | 0 | 0 |
| PETN/fathead minnow | 10 | 2 | 100 | 100 | 100 |
| | 6.5 | 3 | 100 | 100 | 100 |
| | 3.2 | 3 | 0 | 0 | 33 |
| | 1.0 | 2 | 0 | 0 | 0 |
| | 0.65 | 2 | 0 | 0 | 0 |
| | Control | 3 | 0 | 0 | 0 |
| Tetracene/bluegill | 7.5 | 2 | 100 | 100 | 100 |
| | 5.6 | 2 | 100 | 100 | 100 |
| | 4.2 | 2 | 0 | 33 | 67 |
| | 2.1 | 2 | 0 | 0 | 0 |
| | 1.0 | 3 | 0 | 0 | 0 |
| | Control | 2 | 0 | 0 | 0 |
| Tetracene/ fathead minnow | 4.2 | 2 | 100 | 100 | 100 |
| | 3.2 | 2 | 67 | 67 | 100 |
| | 2.4 | 2 | 0 | 0 | 33 |
| | 1.8 | 2 | 0 | 0 | 33 |
| | 1.0 | 2 | 0 | 0 | 0 |
| | Control | 2 | 0 | 0 | 0 |
| Tetracene wastewater/ bluegill | 100 | 2 | 0 | 0 | 0 |
| | 65 | 2 | 0 | 0 | 0 |
| | 32 | 2 | 0 | 0 | 0 |
| | 21 | 2 | 0 | 0 | 0 |
| | 10 | 2 | 0 | 0 | 0 |
| | Control | 2 | 0 | 0 | 0 |
| Tetracene wastewater/ fathead minnow | 100 | 2 | 0 | 0 | 0 |
| | 65 | 2 | 0 | 0 | 0 |
| | 21 | 2 | 0 | 0 | 0 |
| | 10 | 2 | 0 | 0 | 0 |
| | Control | 2 | 0 | 0 | 0 |

Table 7 - Continued

| Compound/Species | Concentration (%, v/v) | N[a] | % mortality observed | | |
|---|---|---|---|---|---|
| | | | 24 hour | 48 hour | 96 hour |
| TNR/bluegill | 14 | 2 | 100 | 100 | 100 |
| | 10 | 2 | 100 | 100 | 100 |
| | 3.2 | 2 | 100 | 100 | 100 |
| | 2.4 | 3 | 0 | 0 | 0 |
| | 1.0 | 3 | 0 | 0 | 0 |
| | Control | 3 | 0 | 0 | 0 |
| TNR/fathead minnow | 14 | 2 | 100 | 100 | 100 |
| | 10 | 2 | 100 | 100 | 100 |
| | 7.5 | 3 | 0 | 0 | 0 |
| | 5.6 | 3 | 0 | 0 | 0 |
| | 4.2 | 3 | 0 | 0 | 0 |
| | Control | 3 | 0 | 0 | 0 |
| TNR wastewater/bluegill | 14 | 2 | 100 | 100 | 100 |
| | 10 | 2 | 100 | 100 | 100 |
| | 7.5 | 3 | 33 | 33 | 33 |
| | 4.9 | 3 | 0 | 0 | 0 |
| | 3.7 | 3 | 0 | 0 | 0 |
| | Control | 3 | 0 | 0 | 0 |
| TNR wastewater/ fathead minnow | 10 | 2 | 100 | 100 | 100 |
| | 7.5 | 2 | 100 | 100 | 100 |
| | 4.9 | 3 | 0 | 33 | 33 |
| | 3.7 | 3 | 0 | 0 | 0 |
| | 2.8 | 3 | 0 | 0 | 0 |
| | Control | 3 | 0 | 0 | 0 |
| FA 956/bluegill | 14 | 2 | 100 | 100 | 100 |
| | 10 | 2 | 50 | 50 | 100 |
| | 7.5 | 2 | 0 | 0 | 33 |
| | 5.6 | 2 | 0 | 0 | 0 |
| | 4.2 | 3 | 0 | 0 | 0 |
| | Control | 3 | 0 | 0 | 0 |
| FA 956/fathead minnow | 10 | 2 | 100 | 100 | 100 |
| | 7.5 | 2 | 33 | 100 | 100 |
| | 5.6 | 2 | 0 | 33 | 67 |
| | 4.2 | 3 | 0 | 0 | 0 |
| | 3.2 | 2 | 0 | 0 | 0 |
| | Control | 3 | 0 | 0 | 0 |

Table 7 - Continued

| Compound/Species | Concentration (%, v/v) | N[a] | % mortality observed | | |
|---|---|---|---|---|---|
| | | | 24 hour | 48 hour | 96 hour |
| FA 956 wastewater/ bluegill | 100 | 2 | 100 | 100 | 100 |
| | 87 | 2 | 100 | 100 | 100 |
| | 75 | 2 | 0 | 0 | 50 |
| | 56 | 2 | 0 | 0 | 0 |
| | Control | 2 | 0 | 0 | 0 |
| FA 956 wastewater/ fathead minnow | 100 | 2 | 100 | 100 | 100 |
| | 75 | 2 | 0 | 100 | 100 |
| | 65 | 2 | 0 | 0 | 0 |
| | 61 | 2 | 0 | 0 | 0 |
| | Control | 2 | 0 | 0 | 0 |

[a]N = number of fish tested/concentration.

Table 8 - Relationship between acute toxicity of neutralized (except tetracene and tetracene wastewater which were tested unneutralized) primer compounds, wastewaters and a reagent blank expressed as percent (volume/volume basis) of primer, wastewater or reagent blank and as milligrams per liter (mg/l) of total solids. Values for water flea based on 48-hour LC50's and for fish, on 96-hour LC50's.

| Compound/Species | LC50 (%, v/v) | LC50 (mg/l, total solids) |
|---|---|---|
| reagent blank/water flea | >1.0<10 | >81<810 |
| " /bluegill | >10<14 | >810<1134 |
| " /fathead minnow | 13.4 | 1085 |
| PbTNR/water flea | 0.160 | 7 |
| " /bluegill | 13.2 | 581 |
| " /fathead minnow | 13.2 | 581 |
| PbTNR wastewater/ water flea | 3.00 | 51 |
| " /bluegill | 8.9 | 151 |
| " /fathead minnow | 10 | 170 |
| PETN/water flea | 0.85 | 292 |
| " /bluegill | >6.5<10 | >2230<3430 |
| " /fathead minnow | 2.7 | 926 |
| Tetracene/water flea | 0.0040 | 0.14 |
| " /bluegill | 3.5 | 123 |
| " /fathead minnow | 1.9 | 66 |
| Tetracene wastewater/water flea | 4.52 | 4.52 |
| " /bluegill | >100 | >100 |
| " /fathead minnow | >100 | >100 |
| TNR/water flea | 0.68 | 52 |
| " /bluegill | >2.4<3.2 | >185<246 |
| " /fathead minnow | >7.5<10 | >578<770 |
| TNR wastewater/water flea | 1.6 | 218 |
| " /bluegill | 7.1 | 966 |
| " /fathead minnow | 5.1 | 694 |
| FA 956/water flea | 0.0029 | 0.2 |
| " /bluegill | 7.4 | 511 |
| " /fathead minnow | 5.4 | 373 |
| FA 956 wastewater/water flea | 13.2 | 92.4 |
| " /bluegill | 70.5 | 490 |
| " /fathead minnow | >65<75 | >455<525 |

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