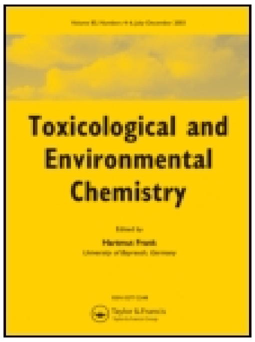


Toxicological & Environmental Chemistry

ISSN: 0277-2248 (Print) 1029-0486 (Online) Journal homepage: https://www.tandfonline.com/loi/gtec20

# Uptake and toxicity of some pesticides on three freshwater fishes (*Oreochromis niloticus*, *Clarias gariepinus* and *Chrysicthys nigrodigitatus*)

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*Toxicological & Environmental Chemistry*, Jan–Mar 2007; 89(1): 111–123

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# Uptake and toxicity of some pesticides on three freshwater fishes (*Oreochromis niloticus*, *Clarias gariepinus* and *Chrysicthys nigrodigitatus*)

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*(Received 25 April 2005; in final form 28 July 2006)*

**Abstract**

Studies were carried out to determine the toxicity of some selected pesticides on fresh water fish in a tropical environment. The uptake of the pesticides lindane, pentachlorophenol (PCP), and propoxur, which are frequently used by farmers and industrialists were studied in concrete ponds at the University of Cape Coast, in Ghana. The fishes used for the study were *Oreochromis niloticus*, *Clarias gariepinus*, and *Chrysicthys nigrodigitatus*, and were obtained from cultured ponds in the Cape Coast District and Mankessim in the Central Region and Weija Dam, in the Greater Accra region of Ghana. Single high lethal concentration (SD) or acute treatment and cumulative/chronic (or multiple minor) lethal concentration (CD) treatment were employed in administering the pesticides to the fishes via water. Gas chromatograph (GC) Electron Capture Detector (ECD) analysis was done on the dead fishes to see the extent of ingestion. The $LC_{50}$ values obtained for lindane on the three fish samples were as follows: *Chrysicthys* – $0.38\,mg\,L^{-1}$; *Oreochromis* – $0.42\,mg\,L^{-1}$, and *Clarias* – $1.2\,mg\,L^{-1}$. Mortalities occurred in fish within 3–5 days of application. For the PCP on *Chrysicthys, Oreochromis*, and *Clarias* species the $LC_{50}$ values were 0.42, 0.32 and $0.64\,mg\,L^{-1}$, respectively for over a 2–3 day period. For a three-time influx period of propoxur the $LC_{50}$ for *Chrysicthys, Oreochromis*, and *Clarias*, were 22.0, 30.40, and 45.04 (all in $mg\,L^{-1}$), respectively. The results obtained indicated that the pesticides had adverse effects on the general growth and reproduction of fishes. Gonadosomatic indices also showed that the pesticides affected the development of the body, the gonads, and their reproduction.

**Keywords:** *Lindane, propoxur, pentachlorophenol,* Oreochromis niloticus*,* Clarias gariepinus*,* Chrysicthys nigrodigitatus*, relative toxicity,* 'Kumakate' *and $LC_{50}$ values*

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ISSN 0277-2248 print/ISSN 1029-0486 online 
DOI: 10.1080/02772240600949931

## Introduction

The misapplication and indiscriminate disposal of lindane, pentachlorophenol (PCP), and propoxur pesticides is gradually approaching crisis level in Ghana. Often, these pesticides are not administered directly to these organisms under study but are affected as a result of drift. Propoxur for instance is used for a good agricultural purpose on Cocoa plants to rid it of insects but has been observed to lead to high mortality of fish in areas where farms are situated near water bodies. Unfortunately, issues of pesticide misapplications are not fully documented in Ghana. Thus it has become difficult to monitor and coordinate the application of these named pesticides so as to thoroughly assess the gravity of the situation. Of particular interest is the use of some banned pesticides, especially the dichlorodiphenyltetrachloroethane (DDT), which is sometimes used in fishing exploits in some villages in Ghana. The siting of some crop farms and fishponds has been done without proper impact assessments. These have further aggravated the environmental crisis, which has become increasingly difficult to deal with.

It has been difficult to assess certain illnesses where the main vector was a fishery product from chemically contaminated fish [1]. It has also been observed that most of these chemicals persist for long periods. Some eventually break down into more harmful metabolites [2].

Too often, pesticide residue levels that may be too low to detect in diets have been found to be concentrated in the breast milk of lactating mothers, in the human blood and in adipose tissue. Several species of fish, invertebrates and algae have shown levels of PCP that were significantly higher (up to 10,000 times) than the concentration in the surrounding waters. This calls for a proper study of the effects of chemicals in humans, animals, and the environment in general.

This research is aimed at generating comprehensive data on the effects of frequently used pesticides when administered to freshwater fish and their environments. In doing this, pesticides were artificially introduced into concrete ponds containing the fishes to mimic the real practices carried out in the open field.

## Materials and methods

Juvenile fish were obtained from ponds in the Central and Greater Accra regions of Ghana and kept in holding tanks for a period of 1 month to eliminate transport-induced stress and allow for capture-induced mortalities. The fish had an average fork length of 4 cm and were purported to be two weeks old, when obtained from the suppliers. Thirty fishes each were kept in each of the experimental and adjoining control ponds. Each pond had an average water holding capacity of 30 L. The design of the ponds ensured no exchange of test materials or solutions. Thus the loading rate was one fish per litre. The pond was situated in the open, behind the University's science complex. Weather conditions remained fair within the experimental period thus negative effects can be excluded as they were minimal. This was confirmed by measurements of water parameters which did not alter much (Table I). Fish were fed on prepared fish feed twice daily.

In the first set of experiments, fish were exposed to an acute single high dose of the named pesticides in their respective ponds, and the immediate effects observed. In the second experiment, which centered on cumulative or chronic studies, different concentrations of the pesticides were administered at periodic intervals within the same experimental pond containing the fish samples. In the case of lindane, the active ingredient, gamma hexachlorocyclohexane, which consists of 99% of the gamma isomer, was used. It had a

Table I. Administration of the pesticides in the ponds for the cumulative (multiple) application (CD) procedure.

| | Lindane ponds | Pentachlorophenol ponds | Propoxur ponds | Control ponds |
|---|---|---|---|---|
| Amounts of pesticides added-(mg/30 L) | 1.07, 2.13, 3.20, 4.27, 5.33 | 1.04, 2.10, 3.11, 4.20, 5.19 | 26.58, 53.16, 79.98, 106.50, 133.29 | None |
| Resulting concentrations ($mg\,L^{-1}$) | 0.0357, 0.071, 0.107, 0.142, 0.178 | 0.035, 0.070, 0.104, 0.140, 0.173 | 0.886, 1.772, 2.666, 3.55, 4.44 | 0 |
| Interval between applications | Weekly | Weekly | Weekly | |
| Duration | | 3 months | | |

Number of replication is 10.

melting point range of 112.5–113.5°C. It had no peculiar smell. It had a solubility of 7.25 $mg\,L^{-1}$ at 20°C and 8.25 $mg\,L^{-1}$ at 25°C. The sodium salt of PCP (sodium pentachlorophenate) was used instead of PCP. Its solubility was 330 $g\,L^{-1}$ at 25°C which is higher than for PCP at 80 $mg\,L^{-1}$ at 30°C. The active ingredient, propoxur was also employed. Propoxur had a solubility of 1.9 $g\,L^{-1}$ at 20°C and a melting point of 90°C. Sodium pentachlorophenate was obtained from SADOFOSS s.a., Cote d'Ivoire, while propoxur was obtained from Bayer Leverkusen in Germany. All other chemicals used were either of the GC or analytical grade supplied by BDH of Merck House, Poole, BD 15 ITD, England.

*Administration of pesticides*

The common pesticides used for this study were lindane ('*Kumakate*'), PCP and propoxur (an active ingredient in Unden and Baygon). Pesticides were administered to the ponds containing the experimental fish. Thus they were imbibed via their gastrointestinal tracts and the surface of their gills and skin.

*Experimental design I – determination of $LC_{50}$ using a single application procedure (SD).* This involved a single high acute test level. In other words an acute exposure was reached by administration of the said pesticide in one single application step (static exposure design). Three replicate ponds containing 30 L of water were spiked with 4.8 mg (0.660 $mg\,L^{-1}$) of Lindane each. Three other replicate ponds also containing 30 L of water were similarly spiked with 3.75 mg (0.125 $mg\,L^{-1}$) of PCP and other sets of 30 L ponds for propoxur were also spiked with 83.25 mg (2.780 $mg\,L^{-1}$) of the propoxur. The effects of each pesticide on the fish were observed and recorded at hourly intervals. The whole experimental set-up was repeated.

The above experiments were repeated with higher doses of the pesticides. The new or higher acute concentrations of pesticides in water, which gave close to 50% mortality (averaged from three fish species), were 0.3, 0.18 and 3.83 $mg\,L^{-1}$ of lindane, PCP, and propoxur respectively.

*Experimental design II – cumulative (multiple) application (CD) procedure.* The second experiment was focussed on chronic cumulative exposure studies. Increasing amounts of pesticides were administered intermittently over a period of 2 months to the same test water in weekly intervals (up to a total of five applications). However, daily observations were made up to the third month.

The pesticides were administered in increasing orders as outlined subsequently so as to create an apparent 'build-up' or cumulative effect. These different quantities of pesticides were administered into the same 30 L of each of the respective ponds without changing the initial water, which already contained the previously applied pesticides.

*Extraction*

Fish that died during the experiment were examined for the tested pesticides.

Lindane was extracted from the dead fishes with 6% ether ($94 \pm 6$ per ether – ether mixture) in a florisil column, while PCP was extracted from the dead fishes with a 4 : 1 hexane $H_2SO_4$ and cleaned finally with hexane. Propoxur on the other hand was extracted from the dead fishes with chloroform.

At each point of experimental analysis a third of the sample size was analyzed. Three of such analyses were carried out for all individual experimental setups.

Concentrations of pesticides imbibed by fish and their $LC_{50}$ values were determined by gas chromatography. Graphical representations also gave vivid pictures of the observations.

## Results and discussion

Figures 1–3 show the comparative effect of lindane on the three fish samples used.

A comparative display of the effect of propoxur on *Chrysicthys, Oreochromis and Clarias* spp. is shown in Figure 4(a)–(c). A more comparative overview of the effects of the acute administrations of pesticides are shown in Figure 4(a)–(c). It shows how each of the three fishes was affected by the test pesticides under study in the acute experiments.

Low- and high-concentrations are for Lindane 0.166/0.300 $mg\,L^{-1}$, PCP 0.125/ 0.183 $mg\,L^{-1}$ and propoxur 2.780/3.830 $mg\,L^{-1}$, respectively. The effect of cumulative or chronic pesticide application on fish mortality is indicated graphically in Figures 5–7.

Comparing the two sets of graphs on acute and chronic toxic exposures, it could be observed that even though approximate amounts of Lindane and PCP were administered the PCP tended to have devastating effects on the fish, especially *Chrysicthys*, even at the low concentration administered. However, in the case of the Lindane, an effect was observed only at high pesticide concentration. The *Clarias* species were the least affected in both cases, while the *Chrysicthys* species was most affected, even at low PCP concentration.

The effect of propoxur was not as devastating though it was administered in a greater quantity, indicating that it had less harmful effect on the fishes.

The accumulation of pesticides was dependant on the type of fish and the periodic 'influxes' of pesticides. Regression analysis of the data showed that the concentration of pesticides, the type of pesticide, the type of fish, and the time of exposure exerted significant effects on the survival of fish, especially on *Chrysicthys* and *Oreochromis* species. The variables observed include: fish *versus* the types of pesticides, fish types *versus* the concentrations of pesticides and changes of the concentrations of pesticides on mortalities.

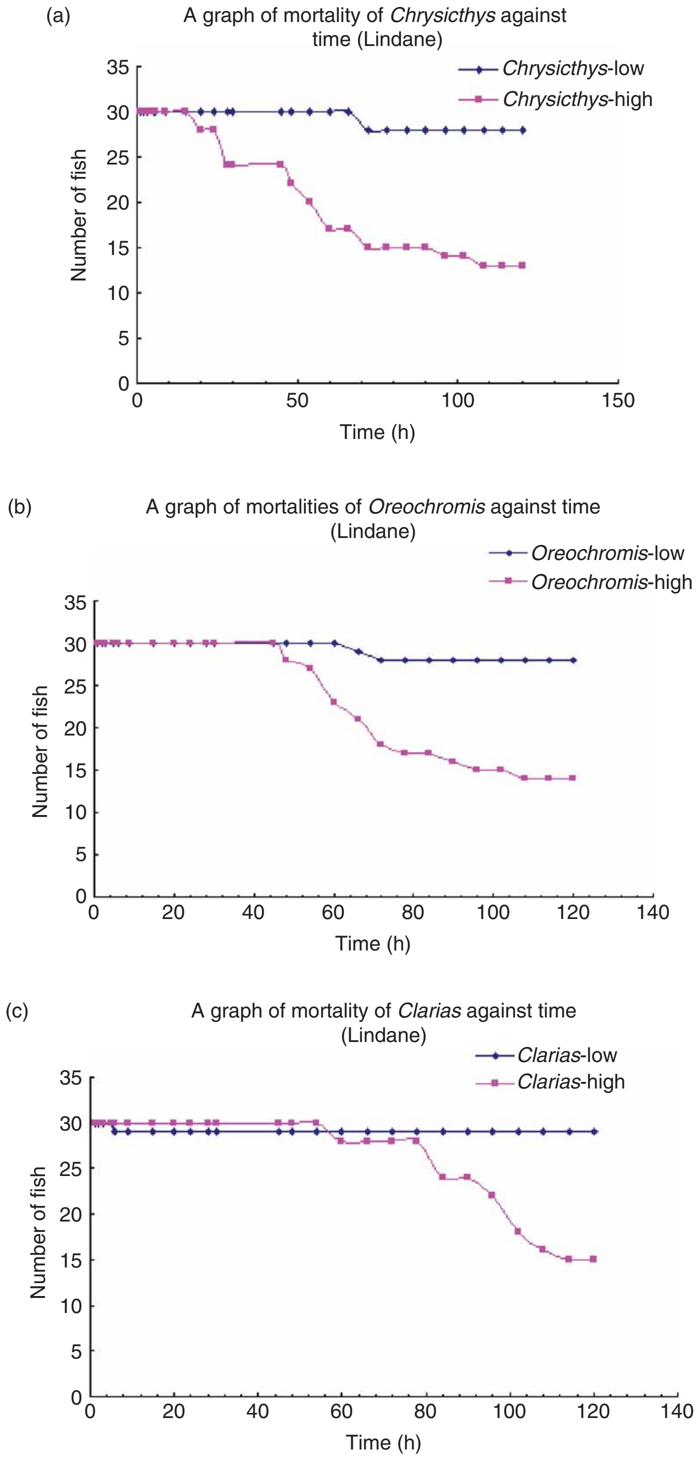
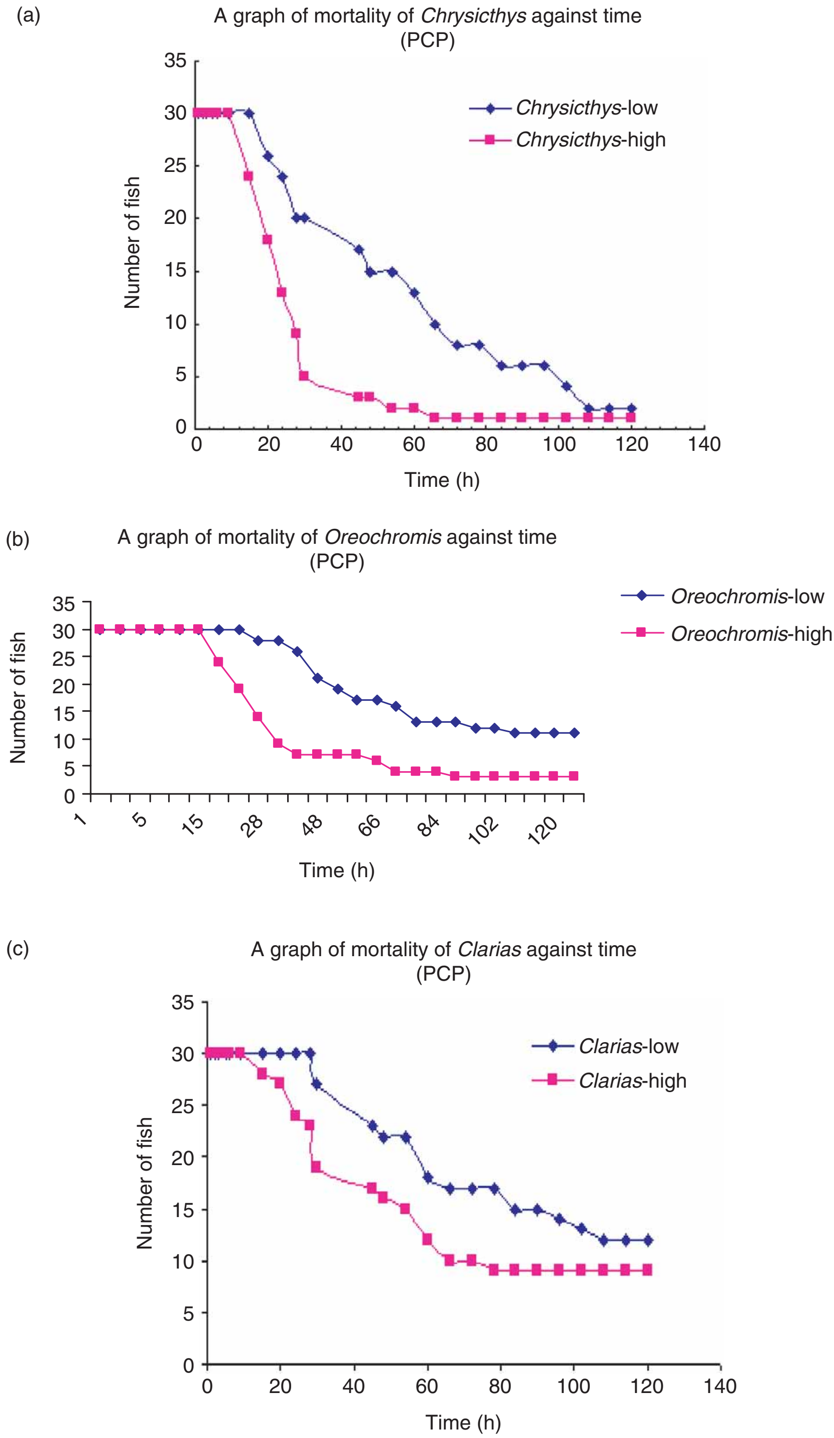



Figure 1a–c. A comparative display of the effects of Lindane on *Chrysisthys*, *Oreochromis and Clarias* spp.




Figures 2a–c. A comparative display of the effects of PCP on *Chrysicthys*, *Oreochromis and Clarias* spp.

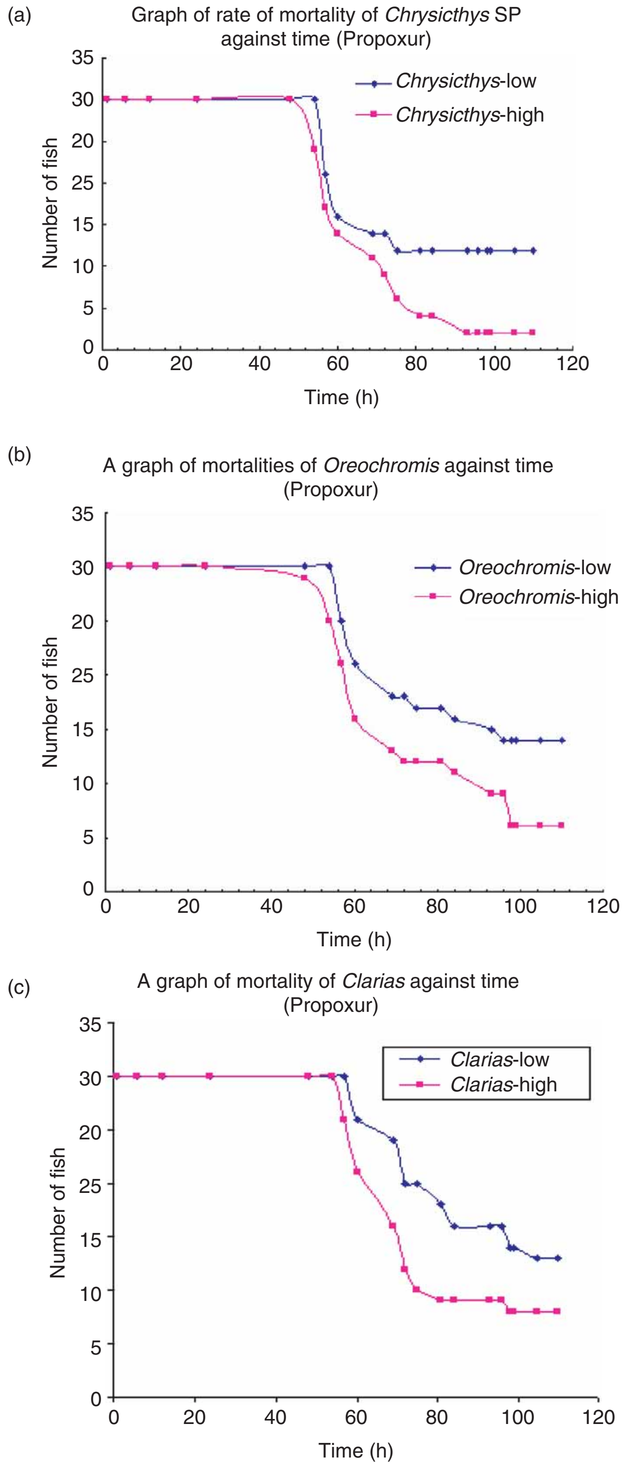



Figure 3a–c. A comparative display of the effect of Propoxur on *Chrysicthys*, *Oreochromis and Clarias* spp.

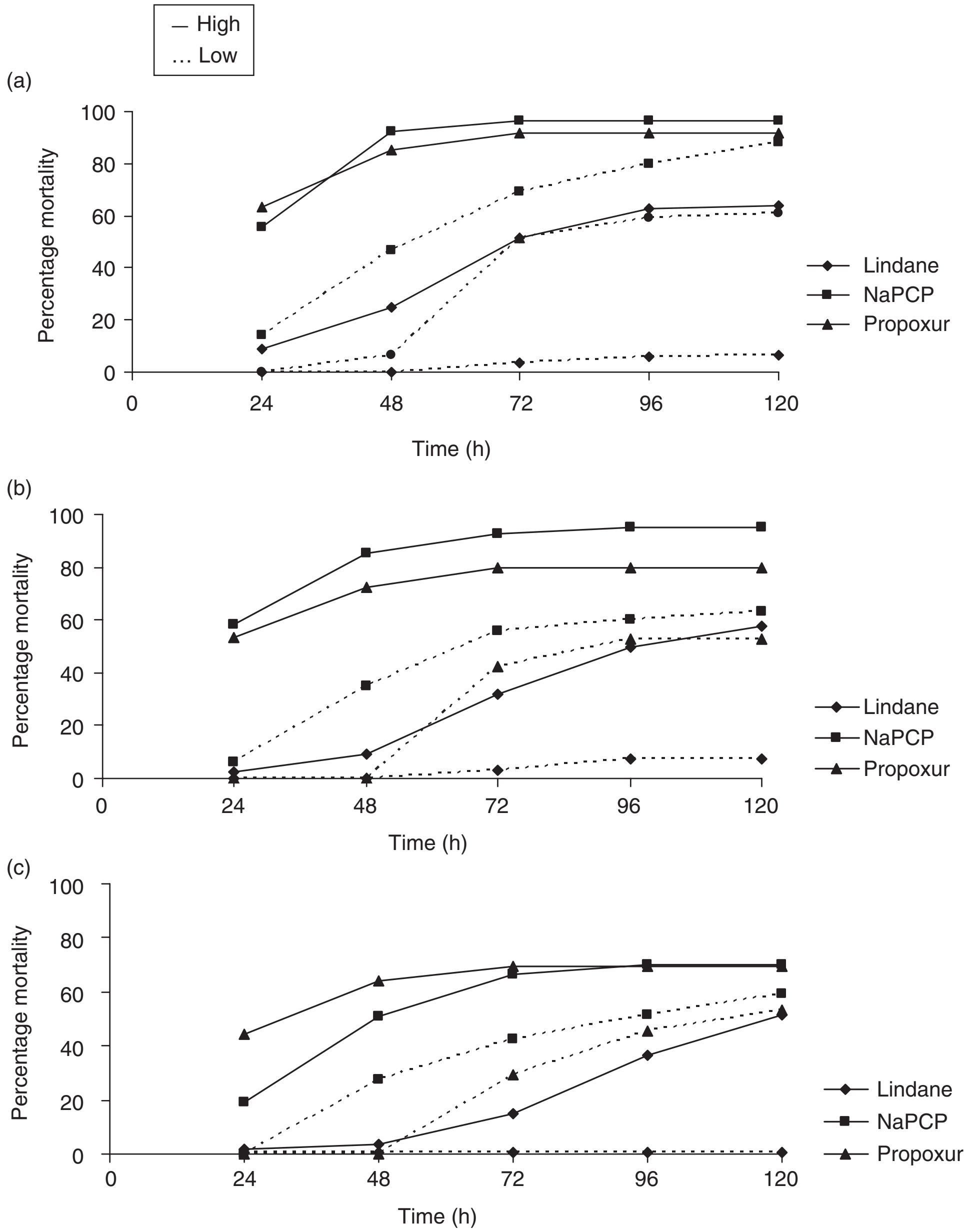



Figure 4. Effect of applied pesticides on *Chrysicthys* (a), *Oreochromis* (b), and *Clarias* spp.

Propoxur, for example, which has replaced DDT in controlling blackflies in Ghana, is toxic to fish as well as to other organisms that eat fish. Its spray drifts and run-offs have been found to be fatal to other non-target organisms after a useful application.

The effect of cumulative pesticide application on fish mortality is indicated above in mortality graphs.

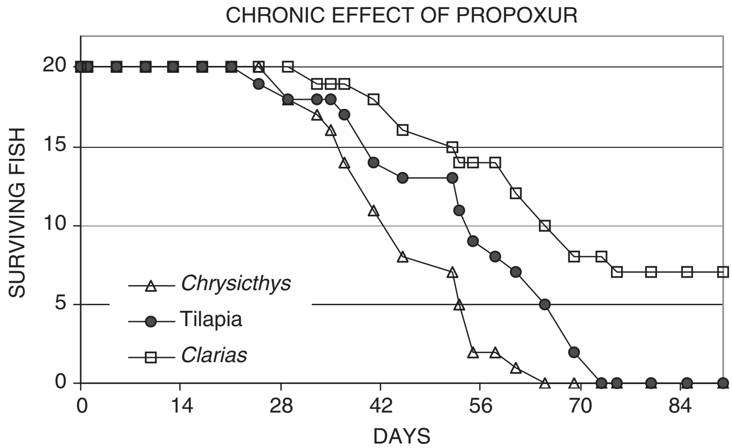



Figure 5. The effect of cumulative or chronic effect of propoxur application on fish survival.

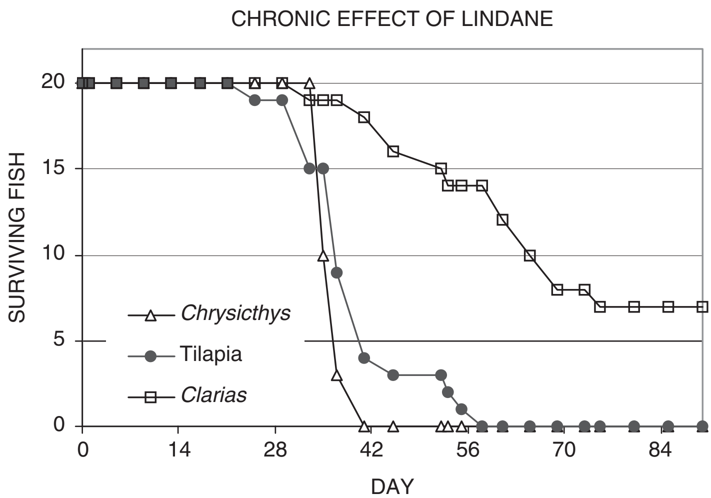



Figure 6. The effect of cumulative or chronic effect of Lindane application on fish survival.

Observed water parameters (temperature, oxygen, chloride, pH, conductivity, and dissolved solid) indicated that changes in the control as compared to the treated ponds did not vary too drastically, and, therefore, changing climatic conditions in the treated waters could not have contributed much to the mortality observed among the fishes (Tables II–IV). However, levels of chloride which resulted from the application of pesticides could have contributed to stress factors.

*Gonodosomatic index analyses.* These studies also enabled us to find out if the pesticides affected the growth of experimental fish in any way. The observed results are shown below.

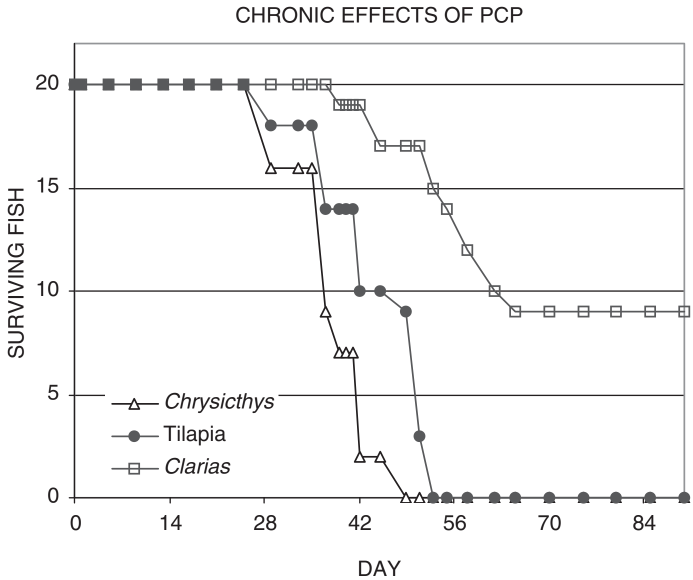



Figure 7. The effect of cumulative or chronic effect of PCP pesticide application on fish mortalities.

Table II. The results of the effects of the applied pesticides on temperature, oxygen, chloride, pH, conductivity, and dissolved solid on the water used.

| Measured parameter | Range for control ponds | Range for Lindane ponds | Range for PCP ponds | Range for Propoxur ponds |
|---|---|---|---|---|
| Temp (°C) | 25–27 | 25.5–27.5 | 25.4–27.7 | 25.6–27.5 |
| Oxygen ($mg\,L^{-1}$) | 8.0–10.0 | 4.0–8.0 | 4.0–8.0 | 4.5–9.0 |
| Chloride per 100 $cm^3$ of $H_2O$ | 2.5–2.8 | 3.1–4.7 | 3.1–4.4 | 3.1–3.6 |
| pH | 7.5–8.5 | 7.0–8.5 | 7.0–8.4 | 7.0–8.8 |
| Electrical conductivity ($ms\,m^{-1}$) | 30–34 | 35–41 | 30–46 | 29–52 |
| Dissolved solids g/100 $cm^3$ | 0.02–0.05 | 0.01–0.08 | 0.01–0.09 | 0.02–0.10 |

Table III. Data on GC results for acute exposure.

| Experiment (SD) | Concentration in water ($mg\,L^{-1}$) | Concentration in *Clarias* sp. ($mg\,kg^{-1}$) | Concentration in *Oreochromis* sp. ($mg\,kg^{-1}$) | Concentration in *Chrysicthys* sp. ($mg\,kg^{-1}$) |
|---|---|---|---|---|
| Lindane, acute exposure −4.8 mg (low) | 0.16 | $9.30 \times 10^2$ (±0.06) | $2.83 \times 10^2$ (±0.03) | $2.02 \times 10^2$ (±0.02) |
| Lindane, exposure −9.0 mg (high) | 0.30 | $1.05 \times 10^3$ (±0.04) | $3.37 \times 10^2$ (±0.03) | $2.69 \times 10^2$ (±0.04) |
| PCP exposure −3.75 mg (low) | 0.125 | $6.00 \times 10^2$ (±0.03) | $2.38 \times 10^2$ (±0.04) | $2.97 \times 10^2$ (±0.04) |
| PCP exposure −5.4 mg (high) | 0.180 | $6.89 \times 10^2$ (±0.02) | $2.85 \times 10^2$ (±0.04) | $3.56 \times 10^2$ (±0.05) |
| Propoxur exposure −83.25 mg (low) | 2.80 | $4.45 \times 10^4$ (±0.01) | $3.38 \times 10^4$ (±0.02) | $2.02 \times 10^4$ (±0.02) |
| Propoxur exposure −115.0 mg (high) | 38.3 | $4.65 \times 10^4$ (±0.03) | $3.55 \times 10^4$ (±0.04) | $2.10 \times 10^4$ (±0.02) |

Table IV. Data on GC results for chronic exposure.

| | Experiments | | | |
|---|---|---|---|---|
| Average chronic exposure (CD) | Concentration in water ($mg\,L^{-1}$) | Concentration in *Clarias* ($mg\,kg^{-1}$) | Concentration in *Oreochromis* ($mg\,kg^{-1}$) | Concentration in *Chrysicthys* ($mg\,kg^{-1}$) |
| Lindane | $1.5\times10^3\pm0.01$ | $1.20\times10^3\pm0.04$ | $1.20\times10^2\pm0.01$ | $1.10\times10^2\pm0.02$ |
| PCP | $7.60\times10^2\pm0.01$ | $6.42\times10^2\pm0.01$ | $3.24\times10^2\pm0.01$ | $3.65\times10^2\pm0.04$ |
| Propoxur | $6.60\times10^4\pm0.01$ | $4.55\times10^4\pm0.02$ | $3.45\times10^4\pm0.01$ | $2.20\times10^4\pm0.01$ |

Table V. A summarized table showing the GSI values for untreated (non-pesticides) fish.

| Type of experiment | Number of samples analyzed | Mean body weight | Sex | Mean GSI | Interpretation |
|---|---|---|---|---|---|
| Untreated/control fish – *Clarias* sp. | 10 | 28.05 | Female | 1.15 | Normal |
| Untreated fish – *Clarias* sp. | 8 | 27.44 | Male | 2.61 | Normal |
| Untreated *Oreochromis* sp. | 11 | 28.70 | Female | 3.83 | Normal |
| Untreated *Oreochromis* sp. | 8 | 22.29 | Male | 0.58 | Normal |
| Untreated *Chrysicthys* sp. | 12 | 29.03 | Female | 3.21 | Normal |
| Untreated *Chrysicthys* sp. | 7 | 25.81 | Male | 0.75 | Normal |

It was observed that body weights and gonad weights of pesticide-induced fish were relatively low, as compared to the weight of control fish. As indicated earlier, GSI (Ig) is another guide to the reproductive state of a fish [3]. Here

$$\mathrm{Ig} = \frac{\mathrm{gW}}{\mathrm{GW}} \times 1000$$

where

$$\mathrm{gW} = \text{gonad weight}$$
$$\mathrm{GW} = \text{gutted weight of fish}$$

The above formula is used in case of large fish, where they may contain large quantities of food, which could lead to a misleading total weight [4].

In the work, 'Aspects of the reproductive biology of the red Pandora, *Pagellus bellottii*' (Pisces: Sparidae) in Ghana [4], the author states,

$$\mathrm{GSI} = \frac{100\mathrm{GW}}{\mathrm{BW} - \mathrm{GW}},$$

GW = gonad weight and BW = body weight as observed in works of Blay Jr and Eyeson. All the above formula, strive to give a co-efficient of maturity – sexual maturity or development. The observations and results obtained from GSI studies are indicated in Tables V–VII.

## Conclusion

Other observed lethal dose and concentration values for other fish species which had been affected by pesticides under study showed a close relation to observed values. The Propoxur

Table VI. GSI values for treated (pesticide treated) fish and their significance.

| | | Mean body weights | Lindane GSI | PCP GSI | Propoxur GSI |
|---|---|---|---|---|---|
| *Clarias* sp. | Males | 20.58 | 0.18 | 0.24 | 0.41 |
| | Females | 16.98 | 2.68 | 3.45 | 3.14 |
| *Oreochromis niloticus* | Males | 18.29 | 0.22 | 0.63 | 0.48 |
| | Females | 18.56 | 2.36 | 3.45 | 2.48 |
| *Chrysicthys* sp. | Males | 24.80 | 1.05 | 0.88 | 1.29 |
| | Females | 21.50 | 3.55 | 3.10 | 3.44 |

Table VII. Summarized statistical evaluation of the gonadosomatic index (GSI) of treated fish in comparison to the untreated fish.

| | | Lindane | PCP | Propoxur |
|---|---|---|---|---|
| *Clarias* sp. | Males | ↓↓ | ↓↓ | ↓↓ |
| | Females | ↓↓ | ↓ | ↓ |
| *Oreochromis niloticus* | Males | ↓↓ | ↑ | No |
| | Females | ↓↓ | ↓ | ↓↓ |
| *Chrysicthys* sp. | Males | No | No | No |
| | Females | No | No | No |

No . . . not statistically significant from the control fish (CI 95%).
↑ . . . Statistically significant increased GSI in treated fish (CI 95%).
↓↓ . . . Statistically significant reduced GSI in treated fish (CI 95% and CI 99%).
↓ . . . Statistically significant reduced GSI in treated fish (CI 95%).

48-h $LC_{50}$ for fathead minnow is 19 ppm, while the 96-hour $LC_{50}$ for rainbow trout is 13.6 $mg\,L^{-1}$.

In the case of PCP, the reported $LC_{50}$ values are 68 $\mu g\,L^{-1}$ in Chinook salmon, 52 $\mu g\,L^{-1}$ in rainbow trout, 205 $\mu g\,L^{-1}$ in fathead minnow, 68 $\mu g\,L^{-1}$ in channel catfish and 32 $\mu g\,L^{-1}$ in bluegill sunfish. Again, data on the effect of PCP and other chlorophenols on *Notropis* sp. showed effects such as reduction in egg hatching and an increase in malformed embryos. This occurred at a concentration of 100 $mg\,L^{-1}$. The same observation was made for *Cyprinus carpio* (common carp) when chlorophenols were applied indirectly. In *Notropis cornutus* (shiner) growth was reduced by 25% when Lindane was applied. Fish survived, however, at a concentration of 0.32 $mg\,L^{-1}$ (The pesticide Manual).

The $LC_{50}$ values obtained for Lindane on the three fish samples were as follows: *Chrysicthys* – 0.38 $mg\,L^{-1}$; *Oreochromis* – 0.42 $mg\,L^{-1}$, and *Clarias* – 1.2 $mg\,L^{-1}$. Mortalities occurred in fish within 3–5 days of application. For the PCP on *Chrysicthys, Oreochromis*, and *Clarias* species the $LC_{50}$ values were 0.42, 0.32, and 0.64 $mg\,L^{-1}$, respectively for over a 2–3 day period. For a three-time influx period of Propoxur the $LC_{50}$ for *Chrysicthys, Oreochromis*, and *Clarias*, were 22.0, 30.40, and 45.04 (all in $mg\,L^{-1}$), respectively.

Of all the fish species, *Clarias gariepinus* was found to be most tolerant to all the pesticides used. *Chrysicthys nigrodigitatus* was the most susceptible to the adverse effects of the pesticides. Graphical analysis showed that PCP was the most potent pesticide, while propoxur had little effect on mortality and growth. Lindane affected the reproductive growth of fishes, as fish treated with this pesticide had the smallest (shrunken) gonads.

The adverse effects on reproductive growth were observed after two months of exposure. However mortalities in the instant (SD) experiment occurred within the first 3 days, while mortalities in the CD experiment occurred after 2 months.

This study has proved that sudden flushes of sewage or pollutants, particularly pesticides could have adverse effects on the life of aquatic organisms, especially fish. Periodic minor trickles, though dangerous, could enable the organisms such as fish to develop some level of tolerance. Long-term consequences though may still be disastrous and may be carried along food chains in cumulative effects.

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