Determination of Median Lethal Concentration (LC$_{50}$) for Endosulfan, Heptachlor and Dieldrin Pesticides to African Catfish, *Clarias gariepinus* and Their Impact on Its Behavioral Patterns and Histopathological Responses

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1. Introduction

Pesticides are widely applied in intensive agricultural and aquaculture settings to manage pest populations such as insects, weeds, mollusks and microbial pathogens. Pesticides can enter aquatic environments through direct applications or through several indirect mechanisms including runoff, drainage and wind drift [1], eventually affecting non-target aquatic organisms such as fish and crustaceans, thereby having considerable...
ecological and economic impacts [2]. Among commonly used pesticides, organochlorines are among the most serious concerns as a result of their persistence in air, sediment and ground water, and their propensity for bioaccumulation in food webs, often resulting in deposition in fish gill, liver, muscle, kidney, stomach and brain tissues [3–5].

These pesticides are associated with a range of carcinogenic, teratogenic and endocrine-disruptive effects in vertebrate organisms, including humans [6]. Organochlorine pesticides (OCPs) are categorized as ‘severely hazardous’ pesticides [7] and toxic to fish at moderate concentrations [8].

Endosulfan, Dieldrin and Heptachlor are highly problematic among organochlorine pesticides. Endosulfan was first registered in the USA during 1954 to manage agricultural mites, insects, and other pests [8]. Endosulfan is a highly persistent organic pollutant with a half-life in water from 3 to around 150 days, depending on the parameters such as pH, turbidity, dissolved oxygen and the presence or absence of aquatic pollutants [9]. Endosulfan is extensively applied in rice paddies in developing countries because of its relatively low cost and high degree of effectiveness, despite official prohibition of its use in developed countries such as the UK, the Netherlands, Sweden, Germany, Singapore and Columbia [10]. Endosulfan starts to exert toxic effects on aquatic organisms even at concentrations as low as 0.005 mg/L and hepatotoxic actions can occur quickly after absorption through skin, lung or stomach [10].

Dieldrin is used worldwide to control pests in agriculture and fish farming [11]. Dieldrin interacts at the neurotransmitter receptor level in the fish nervous systems, as reported in largemouth bass (Micropterus salmoides), causing neurotoxicity in the brain [12,13].

Heptachlor is an organochlorine pesticide extensively used to control termites and other insects in developed countries for the past 30 years [14]. Due to its toxicity to animals and humans, the U.S. and other developed countries have banned its use since 1978 [15]. However, heptachlor is still used for seed treatment and pre-planting agricultural practice by several tropical and subtropical countries [14]. Heptachlor is similar to other OCPs because of its chemical features and the compound persists in the soil or sediment for several years even at trace concentrations in the parts-per-billion (ppb) range [15]. The recorded half-life of heptachlor is around 250 days [16], although residues of heptachlor have been reported in soil 14 to 16 years after application, thereby resulting in groundwater pollution [14]. Oxidation during metabolism by a variety of plants and animals can generate heptachlor epoxide (a metabolite or degradation product of heptachlor) which by comparison is more toxic, and degrades relatively slower [15]. Heptachlor and heptachlor epoxide are both poorly soluble in water, leading to adsorption to sediments and bioaccumulation in aquatic food chains, particularly affecting benthic shellfishes and teleosts [9]. Fatty tissues, liver, muscle and neural structures of fish and shellfishes are vulnerable to heptachlor and heptachlor epoxide accumulation. These compounds have extended half-lives from months to years in lipid-based structures. Heptachlor and its metabolites are particularly toxic in liver [17]. Endosulfan concentration was reported in Snakehead (Channa striatus), Javanese carp (Puntius gonionotus), Gourami (Trichogaster sp.) and Climbing perch (Anabas testudineus) at 0.8–4.8, 1.1–4.8, 0.4–3.9 and 0.4–4.2 ng/g, respectively, from the Peninsular Malaysia rice fields [18]. Dieldrin concentration was observed in Catfish (Arius sp.), Blood cockle (Anadara granosa) and Mullet (Valamugil sp.) at 0.02–0.50, 0.01–0.70 as well as 0.02–0.8 ng/g, respectively, in the Straits of Malacca, Malaysia [18]. Heptachlor concentration was also found in Catfish (Arius sp.), Blood cockle (Anadara granosa) and Mullet (Valamugil sp.) at 0.3–8.2, 0.27–3.54 and 0.1–5.2 ng/g, respectively, from the same area [18]. These reports indicate the presence of endosulfan, dieldrin and heptachlor residues at the rice fields areas in Malaysia.

Fishes are sensitive indicators of environmental contaminants because of their consistent responsiveness to dilute pollutant exposure, many of which measurably disrupt physiological and biochemical mechanisms [19]. Histopathology contributes an efficient means of distinguishing the impact of pollutants in various organs such as a number of lesions in particular organs [20] such as gill [21] and liver [22]. These are considered
convenient organs for histological observation to observe the effect of pollution. Other aquatic organisms contaminated with sublethal concentrations of pesticides may cause histological and physiological alterations in tissues of vital organs [23].

A few studies have been performed on how several endosulfan concentrations affect survival and behavioural abnormalities as well as histopathological responses in the Asian swamp eel, *Monopterus albus* and Climbing perch, *Anabas testudineus* from the Muda rice fields, Malaysia [24,25]. The study revealed that *M. albus* and *A. testudineus* are highly susceptible to endosulfan causing alarming effect on survival, behavioural abnormalities as well as histopathological alterations in several vital organs. However, the toxicity of endosulfan, dieldrin and heptachlor in African catfish, *Clarias gariepinus* (a commercially important species in aquaculture) remains poorly understood.

In Malaysia, *Clarias gariepinus* is the dominant fish species in rice producing regions, ranking as the second highest contributor to aquaculture production, following *Oreochromis niloticus*, the Nile Tilapia [26]. The culturing species may be threatened due to the considerable risk of exposure to agro-chemicals applied in crop-production areas with waste discharges that reach groundwater [27]. Hence, this study aims to improve our knowledge of how endosulfan, dieldrin and heptachlor affect African catfish, *Clarias gariepinus* through the determination of the median lethal concentration (LC$_{50}$) values; investigation of the behavioral abnormalities as well as observation of the histopathological responses of gill, liver and muscle to these compounds.

2. Materials and Methods

This study was conducted at the Wet Lab, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia (UPM), 43,400 UPM, Darul Ehsan, Selangor, Malaysia in full compliance with the ethics protocol of Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM), as evaluated accordingly IACUC ethics approval reference no.: UPM/IACUC/AUP-R019/2021; Dated: 23 July 2021.

2.1. Fish and Acclimation Condition

A total of 665 juvenile (16–17 cm total length) African catfish were placed into a fiberglass tank (water holding capacity 1 ton) for 2 weeks prior to pesticide exposure. Dechlorinated tap water was used as the test medium. The fiberglass tank was outfitted with centralized aeration, using 4 aerated outlets inside the tank and a central drainage system for easy waste removal. Water temperature of the tank was consistent with the surrounding ambient temperature. Fish were given commercial floating pellets (SISO Goldy Color) twice daily to satiation level and approximately 40% of the water was exchanged daily for removal of fecal matters and leftover food particles to ensure optimal water quality [28]. The water quality parameters were maintained following the fish acute toxicity testing guidelines (OECD Test Guideline No. 203) [29]. During acclimation, the mortality rate was ≤2%. From the acclimatized juvenile stock, a total of 648 juveniles (324 juveniles for the range test and 324 juveniles for final test) were selected for determination of the median lethal concentration.

2.2. Preparation of Aquariums and Stocking of Tests Fish

In this case, 18 aquariums (76 cm × 38 cm × 38 cm) with 40 L capacity were prepared for experimental exposure to each pesticide, with three units for each concentration. For avoidance of the fungal infection, the glass aquaria were cleaned with 1% potassium permanganate (KMnO$_4$) and therefore sun dried before the exposure tests. Before stocking, fish were screened for any pathogenic infections. The active and healthy fish were placed into the glass aquaria containing dechlorinated tap water. Six (06) individuals were released in each aquarium to avoid the development of maladaptive behavior to improve animal welfare [30]. The aquaria were equipped with aeration system to ensure optimum oxygen level [31] where 12 h light and 12 h dark (LD 12:12) photoperiod was maintained [29].
2.3. Exposure of Contaminants and Determination of LC$_{50}$ (Range Test and Final Test)

The exposure test was a static (i.e., the pesticides concentrations were kept constant) based test where endosulfan and dieldrin based pesticide solutions were made with a concentration of 0 (no pesticides, only n-hexane solvent mixed here as control), 0.001, 0.001, 0.01, 0.1 and 1 mg/L for range test. In the range test of endosulfan exposure, 100% mortality of test fish occurred within 24-h when the concentration was 1 mg/L. The mortality was 33% of the test fish at the concentration of 0.001 mg/L and at the concentration level of 0.01 mg/L, the mortality rate of the test fish was 67%. So, the final experiment for median lethal concentration of endosulfan exposure was carried out at concentration levels ranging from 0.001 to 0.01 mg/L. The range test results showed that the LC$_{50}$ of endosulfan was within 0.001 to 0.019 mg/L which is presented in Table 1. In the range test for dieldrin exposure, 100% mortality of the test fish happened within 24-h at the concentration of 1 mg/L. Percentage mortality of test fish in range test for the different concentrations of dieldrin is given in Table 2. The dieldrin concentration level of 0.001 mg/L caused 33% mortality of the test fish and the concentration 0.1 mg/L obtained 83% average mortality of the test fish (Clarias gariepinus). So, the concentration levels within 0.001 to 0.1 mg/L were taken into consideration for the final test to determine the median lethal concentration of dieldrin exposure to the test fish (C. gariepinus). The range test reported that the median lethal concentration (LC$_{50}$) of dieldrin was within 0.001 to 0.032 mg/L.

Table 1. Range test for (%) mortality response of test fish to the exposure of endosulfan.

| Exposure Conc. (mg/L) | Initial No. of Test Fish | Count of Dead Fish with the Time of Exposure | % Mortality Response (avg.) |
|-----------------------|--------------------------|---------------------------------------------|-----------------------------|
| 0                     | 18                       | 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 | 0                           |
| 0.0001                | 18                       | 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 1 | 17                          |
| 0.001                 | 18                       | 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 3 | 33                          |
| 0.01                  | 18                       | 0 SIM 3 SIM 3 SIM 3 SIM 3 SIM 3 SIM 3 SIM 3 | 67                          |
| 0.1                   | 18                       | 0 SIM 12 SIM 3 SIM 3 SIM 3 SIM 3 SIM 3 SIM 15 | 83                          |
| 1                     | 18                       | 18 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 18 | 100                         |

Table 2. Range test for (%) mortality response of test fish to the exposure of dieldrin.

| Exposure Conc. (mg/L) | Initial No. of Test Fish | Count of Dead Fish with the Time of Exposure | % Mortality Response (avg.) |
|-----------------------|--------------------------|---------------------------------------------|-----------------------------|
| 0                     | 18                       | 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 | 0                           |
| 0.0001                | 18                       | 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 3 | 17                          |
| 0.001                 | 18                       | 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 3 | 33                          |
| 0.01                  | 18                       | 0 SIM 3 SIM 3 SIM 3 SIM 3 SIM 3 SIM 3 SIM 3 | 67                          |
| 0.1                   | 18                       | 0 SIM 6 SIM 6 SIM 6 SIM 6 SIM 6 SIM 6 SIM 15 | 83                          |
| 1                     | 18                       | 18 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 0 SIM 18 | 100                         |

Then for the final test of endosulfan and dieldrin the solutions were made with a concentration of 0 (no pesticides, only n-hexane solvent mixed here as control), 0.001, 0.002, 0.004, 0.008 and 0.016 mg/L [32].

Heptachlor based pesticide solution was prepared with a concentration of 0 (no pesticides, only n-hexane solvent mixed here as control), 0.002, 0.02, 0.2, 1 and 2 mg/L for the range test. In the range test of heptachlor, the concentration of 2 mg/L resulted 100% mortality of test fish within 24 h. Exposure to the concentration of 0.02 mg/L resulted 33% mortality of the test fish, and at the concentration level of 1 mg/L, 83% mortality rate of the test fish was observed within 96 h exposure (Table 3). So, the final test for median lethal concentration of heptachlor was carried out considering the concentration levels ranged from 0.02 to 1 mg/L. The range test result demonstrated that the LC$_{50}$ value of heptachlor was within 0.005 to 0.207 mg/L.

After that pesticide solutions were made with a concentration of 0 (no pesticides, only n-hexane solvent mixed here as control), 0.02, 0.04, 0.08, 0.16 and 0.32 mg/L for the final test [32]. For each pesticide treatment, the individual concentration was subjected to three replications. The analytical grade endosulfan, dieldrin and heptachlor were exposed in this experiment which obtained from SIGMA-Aldrich, Germany through JM Instrument
and Chemical Supply, Kajang, Selangor, Malaysia. The purity of endosulfan, dieldrin and heptachlor was more than 98–99%. For preparing the stock solution of the pesticides, 1 mL n-hexane was used to dissolve the pesticides homogenously. During the exposure of pesticide, no feeding was carried out [32]. The exposure period was 96-h for the test fish, *Clarias gariepinus*. Fish were examined after 2 ± 0.5 h, 5 ± 1 h and 24 ± 2 h from the exposure of pesticides (day 0–1). During the days 2–4 of the examination, all aquaria with living fish were investigated twice per day at early morning and late afternoon. The mortality rate was observed as the number of dead test fish once every 12 h, and then calculated on a cumulative basis for 96-h [29]. The mortality percentage was calculated from the number of dead fish divided by the total number of test fish for each treatment level [33].

| Exposure Conc. (mg/L) | Initial No. of Test Fish | Count of Dead Fish with the Time of Exposure | Cumulative Count of Dead Fish within 96-h Exposure Time | % Mortality Response (avg.) |
|-----------------------|--------------------------|---------------------------------------------|--------------------------------------------------------|----------------------------|
| 0                     | 18                       | 0 0 0                                       | 0 0 0 0                                                 | 0 0                        |
| 0.002                 | 18                       | 0 0 0                                       | 0 0 3 3                                                 | 3 17                       |
| 0.02                  | 18                       | 0 0 0                                       | 3 3 6 12                                                | 6 33                       |
| 0.2                   | 18                       | 0 0 0                                       | 6 6 12 15                                               | 12 67                      |
| 1                     | 18                       | 6 6 3                                       | 15 18                                                   | 83 100                     |
| 2                     | 18                       | 18 0 0                                      | 18                                                      | 100                        |

Critical range tests were carried out to determine the concentrations which causing 50% mortality as used in the final test [30].

2.4. Water Quality during Experiments

Water quality was ensured throughout the experiment. Dissolved oxygen (DO), temperature, pH, electrical conductivity (EC) and total dissolved solid (TDS) of the test media (water) were monitored using Multimeter (YSI) to ensure optimal water quality [32]. The water quality parameters are presented in Table 4 which were monitored after 24-h of exposure of the endosulfan, dieldrin and heptachlor.

| Compounds   | Conc. (mg/L) | Tem. (°C) | DO (mg O₂/L) | pH      | TDS (mg/L) | EC (mS/cm) |
|-------------|--------------|-----------|--------------|---------|------------|------------|
| Endosulfan  | 0            | 25.60 ± 0.10 | 6.15 ± 0.02  | 6.63 ± 0.03 | 0.301      | 0.193      |
|             | 0.001        | 25.60 ± 0.20 | 6.14 ± 0.01  | 6.68 ± 0.33 | 0.301      | 0.193      |
|             | 0.002        | 25.74 ± 0.16 | 6.10 ± 0.04  | 6.73 ± 0.38 | 0.301      | 0.193      |
|             | 0.004        | 25.79 ± 0.22 | 6.09 ± 0.02  | 6.75 ± 0.30 | 0.301      | 0.193      |
|             | 0.008        | 25.84 ± 0.13 | 6.05 ± 0.01  | 6.78 ± 0.30 | 0.301      | 0.192      |
|             | 0.016        | 25.85 ± 0.26 | 5.93 ± 0.05  | 6.84 ± 0.14 | 0.301      | 0.192      |
|             | 0            | 25.50 ± 0.15 | 6.21 ± 0.02  | 6.65 ± 0.03 | 0.299      | 0.186      |
|             | 0.001        | 25.60 ± 0.20 | 6.17 ± 0.03  | 6.68 ± 0.13 | 0.299      | 0.188      |
| Dieldrin    | 0.002        | 25.64 ± 0.16 | 6.16 ± 0.04  | 6.76 ± 0.21 | 0.299      | 0.189      |
|             | 0.004        | 25.76 ± 0.20 | 6.13 ± 0.02  | 6.78 ± 0.30 | 0.300      | 0.192      |
|             | 0.008        | 25.78 ± 0.12 | 6.09 ± 0.01  | 6.80 ± 0.03 | 0.301      | 0.193      |
|             | 0.016        | 25.86 ± 0.23 | 5.98 ± 0.02  | 6.83 ± 0.12 | 0.301      | 0.193      |
|             | 0            | 25.65 ± 0.12 | 6.18 ± 0.02  | 6.65 ± 0.05 | 0.296      | 0.183      |
|             | 0.02         | 25.68 ± 0.02 | 6.16 ± 0.01  | 6.67 ± 0.32 | 0.296      | 0.187      |
| Heptachlor   | 0.04         | 25.72 ± 0.06 | 6.12 ± 0.03  | 6.71 ± 0.18 | 0.297      | 0.190      |
|             | 0.08         | 25.78 ± 0.12 | 6.09 ± 0.05  | 6.75 ± 0.30 | 0.300      | 0.191      |
|             | 0.16         | 25.82 ± 0.16 | 6.08 ± 0.04  | 6.77 ± 0.03 | 0.301      | 0.193      |
|             | 0.32         | 25.87 ± 0.27 | 5.95 ± 0.05  | 6.81 ± 0.11 | 0.301      | 0.193      |

Although temperature and pH play a vital role on the toxicity performance of pesticides [20,34–36], in our study the water quality parameters remained within acceptable limits and only toxic effects of the pesticides were apparent here.
2.5. Investigation of the Behavioural Abnormalities of Fish

The behavioral alterations of the test fish were examined during the exposure of endosulfan, dieldrin and heptachlor for 24–96 h. The behavioral alterations are associated with the physiological responses which are indicative of stress [37]. Pesticide exposure not only results in mortality, but may also result in behavioral abnormalities at sub-lethal pesticide concentrations [30,38]. The behavioral abnormalities of fish such as hyperactivity, jerky movement, abnormal swimming behavior, loss of equilibrium, abnormal ventilatory function, mucus secretion and abnormal skin pigmentation were monitored at a six-hour interval through visual inspection and video recordings during the 96-h exposure of heptachlor, dieldrin and endosulfan (OECD Test Guideline No. 203) [29]. The behavioral abnormalities were categorized as ‘mild’, ‘moderate’ and ‘severe’ where ‘mild’ indicated 30 to 35% of individuals showed the abnormalities; ‘moderate’ indicated 45 to 50% of individuals showed the abnormalities and ‘severe’ indicated 60 to ≥70% of individuals showed the behavioural abnormalities, respectively [11].

2.6. Study of Histopathological Responses

At the end of pesticide exposure period, tissues were dissected from moribund fish resulted from the exposure of LC$_{50}$ dose only and processed for histopathological examination. Control tissues were dissected from the live fish of control aquarium. Samples from the liver, muscle (muscle was dissected from the skin flap under the dorsal fin) and gills were fixed in 10% neutral buffer formalin and were stained with hematoxylin and eosin [39]. The resolutions of the picture were adjusted at 40× magnification. The slides were examined and the histopathological alterations of hepatocytes, sinusoids, hepatic portal vein and red blood cells (RBCs) for the liver; epithelial cells, primary lamellae and secondary lamellae for the gill; epidermis, myotomes and septum for the muscle tissues were captured through light microscope, Motic-BA410, USA (obtained from San Antonio, Schertz, TX, USA) equipped with camera (Moticam pro) and the software MIP (Microscopic Image Processing).

2.7. Statistical Analysis

The 96-h LC$_{50}$ values were estimated with Probit Analysis, using SPSS (version 20.0; SPSS, Chicago, IL, USA) through the dose-response relationship of fish mortality due to the exposure to endosulfan, dieldrin and heptachlor.

3. Results

3.1. Median Lethal Concentration (LC$_{50}$) for 96 h Exposure to Endosulfan through Dose-Response Test

The dose-response test found no mortality at control (0 mg/L) during 96-h exposure period. Percentage mortality of test fish in different concentrations of endosulfan is presented in Table 5. The resulting 96-h LC$_{50}$ value for endosulfan was 0.004 mg/L which resulted in 50% mortality of the test fish.

Table 5. Median lethal concentration (LC$_{50}$) of endosulfan after 96-h exposure to the test fish.

| Exposure Conc. (mg/L) | Total Test Fish | No. of Dead Fish Replicate | Total No. of Dead Fish | % Mortality | LC$_{50}$ (mg/L) |
|-----------------------|-----------------|-----------------------------|------------------------|-------------|------------------|
| 0                     | 18              | 0                           | 0                      | 0           | 0                |
| 0.001                 | 18              | 2                           | 1                      | 3           | 63               |
| 0.002                 | 18              | 2                           | 2                      | 3           | 38               |
| 0.004                 | 18              | 4                           | 2                      | 3           | 50 (0.001–0.01)  |
| 0.008                 | 18              | 4                           | 4                      | 4           | 61               |
| 0.016                 | 18              | 4                           | 5                      | 4           | 72               |

During the Probit analysis, Chi-Square value, $\chi^2$ was 0.095 and no heterogeneity factor was observed at 95% confidence limits. Coefficient of determination value, $R^2$ was 0.98 as well as the lower confidence limit (LCL) was 0.001 and upper confidence limit (UCL) was 0.01, at 95% confidence limit.
3.2. Median Lethal Concentration ($LC_{50}$) for 96-h Exposure to Dieldrin through Dose-Response Test

In the dose-response test, no mortality was found at control (0 ppm) during 96-h exposure period. The percentage mortality of test fish in the dose-response test of dieldrin is presented in Table 6. After 96-h exposure to dieldrin, the resulting $LC_{50}$ value was 0.006 mg/L.

Table 6. Median lethal concentration ($LC_{50}$) of dieldrin after 96-h exposure to the test fish.

| Exposure Conc. (mg/L) | Total Test Fish | No. of Dead Fish | % Mortality | LC50 (mg/L) |
|-----------------------|-----------------|-----------------|-------------|-------------|
| 0                     | 18              | 0               | 0           | 0           |
| 0.001                 | 18              | 2               | 1           | 6           | 0.006 |
| 0.002                 | 18              | 3               | 3           | 7           | 0.006 |
| 0.004                 | 18              | 2               | 3           | 8           | 0.006 |
| 0.008                 | 18              | 3               | 4           | 9           | 0.006 |
| 0.016                 | 18              | 4               | 4           | 11          | 0.006 |

During the Probit analysis, Chi-Square value, $\chi^2$ was 0.084 and no heterogeneity factor was observed at 95% confidence limits, and the Coefficient of determination value, $R^2$ was 0.98.

3.3. Median Lethal Concentration ($LC_{50}$) for 96-h Exposure to Heptachlor through Dose-Response Test

The dose-response test revealed that there was no mortality at control (0 mg/L) during the 96-h exposure period. Percentage mortality of test fish against different concentrations of heptachlor is displayed in Table 7. The resulting 96-h $LC_{50}$ value for heptachlor was 0.057 mg/L.

Table 7. Median lethal concentration ($LC_{50}$) of heptachlor after 96-h exposure to the test fish.

| Exposure Conc. (mg/L) | Total Test Fish | No. of Dead Fish | % Mortality | LC50 (mg/L) |
|-----------------------|-----------------|-----------------|-------------|-------------|
| 0                     | 18              | 0               | 0           | 0           |
| 0.02                  | 18              | 2               | 2           | 6           | 0.056 |
| 0.04                  | 18              | 3               | 3           | 9           | 0.056 |
| 0.08                  | 18              | 3               | 4           | 10          | 0.056 |
| 0.16                  | 18              | 4               | 4           | 11          | 0.056 |
| 0.32                  | 18              | 4               | 5           | 13          | 0.056 |

During the Probit analysis, Chi-Square value, $\chi^2$ was 0.283 and no heterogeneity factor was observed at 95% confidence limits. The Coefficient of determination value, $R^2$ was 0.96 as well as the lower confidence limit (LCL) was 0.006 and upper confidence limit (UCL) was 0.144, at 95% confidence limit.

3.4. Behavioural Abnormalities of Test Fish during the Exposure to Pesticides

No behavioral abnormalities were observed in the control fish. Fish treated with endosulfan at the concentration level from 0.001 to 0.016 mg/L (presented in Table 8), exposed to dieldrin at the concentration level from 0.001 to 0.016 mg/L (presented in Table 9) as well as fish exposed to heptachlor at a concentration level from 0.02 to 0.32 mg/L (demonstrated in Table 10) exhibited mild (around 30 to 35% of individuals showing abnormalities) to moderate (around 45 to 50% of individuals showing abnormalities) behavioral responses for the initial 48 h but afterwards fish started to show severe (around 60 to ≥70% of individuals showing abnormalities) behavioral reactions in terms of hyperactivity, jerky movement, abnormal swimming, disability of equilibrium, abnormalities in ventilatory function, mucus secretion as well as abnormalities in skin pigmentation. In most cases, fish treated to higher concentrations of the pesticides displayed severe abnormal behavior such as very fast swimming, jumping and displaying instability with severe jerky movements, speedy opercular movement, hyperexcitation, and surfacing with gulping of air.
### Table 8. Behavioural abnormalities of *Clarias gariepinus* due to the exposure to endosulfan within the 96-h observation period.

| Conc. (mg/L) | Hyper Activity | Jerky Movement | Abnormal Swimming Behaviour | Loss of Equilibrium | Abnormal Ventilatory Function | Mucus Secretion | Abnormal Skin Pigmentation |
|--------------|----------------|----------------|-----------------------------|---------------------|-------------------------------|----------------|--------------------------|
|              | 24 h            |                |                             |                     |                               |                |                           |
| Control      | -               | -              | -                           | -                   | -                             | -              | -                        |
| 0.001        | -               | -              | +                           | -                   | -                             | -              | -                        |
| 0.002        | +               | -              | +                           | -                   | -                             | -              | -                        |
| 0.004        | +               | -              | +                           | -                   | -                             | -              | -                        |
| 0.008        | +               | +              | +                           | +                   | +                             | +              | +                        |
| 0.016        | +               | +              | ++                          | +                   | +                             | +              | +                        |
| 48 h         |                |                |                             |                     |                               |                |                          |
| Control      | -               | -              | -                           | -                   | -                             | -              | -                        |
| 0.001        | +               | +              | +                           | +                   | +                             | +              | +                        |
| 0.002        | ++              | +              | ++                          | ++                  | ++                            | +              | +                        |
| 0.004        | ++              | ++             | ++                          | ++                  | ++                            | +              | +                        |
| 0.008        | +++             | +++            | +++                         | +++                 | +++                            | +              | +                        |
| 96 h         |                |                |                             |                     |                               |                |                          |
| Control      | -               | -              | -                           | -                   | -                             | -              | -                        |
| 0.001        | +               | +              | +                           | +                   | +                             | +              | +                        |
| 0.002        | ++              | +              | ++                          | ++                  | ++                            | +              | +                        |
| 0.004        | ++              | ++             | ++                          | ++                  | ++                            | +              | +                        |
| 0.008        | +++             | +++            | +++                         | +++                 | +++                            | +              | +                        |
| 0.016        | +++             | +++            | +++                         | +++                 | +++                            | +              | +                        |

'-' indicates no behavioural abnormality; '+' indicates number of individuals showing the behavioural abnormalities; '+' = Mild (around 30 to 35% of individuals showing the behavioural abnormalities); '++' = Moderate (around 45 to 50% of individuals showing the behavioural abnormalities) and '+++' = Severe (around 60 to ≥70% of individuals showing the behavioural abnormalities).

### Table 9. Behavioural abnormalities of *Clarias gariepinus* due to the exposure to dieldrin within the 96-h observation period.

| Conc. (mg/L) | Hyper Activity | Jerky Movement | Abnormal Swimming Behaviour | Loss of Equilibrium | Abnormal Ventilatory Function | Mucus Secretion | Abnormal Skin Pigmentation |
|--------------|----------------|----------------|-----------------------------|---------------------|-------------------------------|----------------|--------------------------|
|              | 24 h            |                |                             |                     |                               |                |                           |
| Control      | -               | -              | -                           | -                   | -                             | -              | -                        |
| 0.001        | -               | -              | +                           | -                   | -                             | -              | -                        |
| 0.002        | +               | -              | +                           | -                   | -                             | -              | -                        |
| 0.004        | +               | -              | +                           | -                   | -                             | -              | -                        |
| 0.008        | +               | +              | +                           | +                   | +                             | +              | +                        |
| 0.016        | +               | +              | ++                          | +                   | +                             | +              | +                        |
| 48 h         |                |                |                             |                     |                               |                |                          |
| Control      | -               | -              | -                           | -                   | -                             | -              | -                        |
| 0.001        | +               | +              | +                           | +                   | +                             | +              | +                        |
| 0.002        | ++              | +              | ++                          | ++                  | ++                            | +              | +                        |
| 0.004        | ++              | ++             | ++                          | ++                  | ++                            | +              | +                        |
| 0.008        | +++             | +++            | +++                         | +++                 | +++                            | +              | +                        |
| 96 h         |                |                |                             |                     |                               |                |                          |
| Control      | -               | -              | -                           | -                   | -                             | -              | -                        |
| 0.001        | +               | +              | +                           | +                   | +                             | +              | +                        |
| 0.002        | ++              | +              | ++                          | ++                  | ++                            | +              | +                        |
| 0.004        | ++              | ++             | ++                          | ++                  | ++                            | +              | +                        |
| 0.008        | +++             | +++            | +++                         | +++                 | +++                            | +              | +                        |
| 0.016        | +++             | +++            | +++                         | +++                 | +++                            | +              | +                        |

'-' indicates no behavioural abnormality; '+' indicates number of individuals showing abnormalities; '+' = Mild (around 30 to 35% of individuals showing the behavioural abnormalities); '++' = Moderate (around 45 to 50% of individuals showing the behavioural abnormalities) and '+++' = Severe (around 60 to ≥70% of individuals showing the behavioural abnormalities).
Table 10. Behavioural abnormalities of *Clarias gariepinus* due to the exposure to heptachlor within the 96-h observation period.

| Conc. (mg/L) | Hyper Activity | Jerky Movement | Abnormal Swimming Behaviour | Loss of Equilibrium | Abnormal Ventilatory Function | Mucus Secretion | Abnormal Skin Pigmentation |
|--------------|----------------|----------------|----------------------------|---------------------|-----------------------------|----------------|---------------------------|
| Control      | -              | -              | -                          | -                   | -                           | -              | -                         |
| 0.02         | -              | -              | +                          | -                   | -                           | -              | -                         |
| 0.04         | +              | +              | +                          | -                   | -                           | +              | -                         |
| 0.08         | +              | +              | +                          | +                   | +                           | +              | -                         |
| 0.16         | +              | +              | +                          | +                   | +                           | +              | -                         |
| 0.32         | +              | +              | +                          | +                   | +                           | +              | -                         |
| Control      | -              | -              | -                          | -                   | -                           | -              | -                         |
| 0.02         | +              | +              | +                          | -                   | -                           | +              | -                         |
| 0.04         | +              | +              | +                          | -                   | -                           | +              | -                         |
| 0.08         | +              | +              | +                          | +                   | +                           | +              | -                         |
| 0.16         | ++             | ++             | ++                         | ++                  | ++                          | ++             | +                        |
| 0.32         | +++            | +++            | +++                        | +++                 | +++                         | +++            | +++                      |
| Control      | -              | -              | -                          | -                   | -                           | -              | -                         |
| 0.02         | +              | +              | +                          | -                   | -                           | +              | -                         |
| 0.04         | +              | +              | +                          | -                   | -                           | +              | -                         |
| 0.08         | +              | +              | +                          | +                   | +                           | +              | -                         |
| 0.16         | ++             | ++             | ++                         | ++                  | ++                          | ++             | +                        |
| 0.32         | +++            | +++            | +++                        | +++                 | +++                         | +++            | +++                      |

'-' indicates no behavioural abnormality; '+' indicates number of individuals showing the behavioural abnormalities; '+' = Mild (around 30 to 35% of individuals showing abnormalities); '++' = Moderate (around 45 to 50% of individuals showing the behavioural abnormalities) and '++++' = Severe (around 60 to ≥70% of individuals showing the behavioural abnormalities).

3.5. Histopathological Responses of Liver, Gill and Muscle Tissues of *Clarias gariepinus* Due to the 96-h Exposure to Endosulfan, Dieldrin and Heptachlor

3.5.1. Histopathological Transformations in Liver

The histopathological transformations in the liver of African catfish due to the 96-h exposure to endosulfan, dieldrin and heptachlor are summarized in Figure 1a–d. Fish in the control group (unexposed) showed typical structures of liver including hepatocytes (polygonal in shape with a prominent nucleus), sinusoids (fenestrated), regular hepatic portal vein and red blood cells [Figure 1a]. Vacuolization in hepatocytes, hepatocytes fusion, melanomacrophages center, pyknotic nuclei along with congestion of RBCs (red blood cells) in HPV (hepatic portal vein) of hepatocytes are reported for the exposure to dieldrin (0.006 mg/L) after 96 h [Figure 1b]. Vacuolization in hepatocytes, disintegration of hepatocytes cell membrane with oozing of cytoplasmic content, pyknotic nuclei as well as congestion of RBCs (red blood cells) in HPV (hepatic portal vein) of hepatocytes are reported for the exposure to endosulfan (0.004 mg/L) after 96 h [Figure 1c]. Congestion of RBCs (red blood cells) in HPV (hepatic portal vein) of hepatocytes, pyknotic nuclei and disintegration of hepatocytes cell membrane along with oozing of cytoplasmic content were observed after 96-h exposure to heptachlor (0.057 mg/L) in *Clarias gariepinus* [Figure 1d].

3.5.2. Histopathological Responses in Gill

The histopathological alterations in the gills of African catfish are illustrated in Figure 2a–d. After 96-h exposure to endosulfan, dieldrin and heptachlor. Fish in the unexposed group (control) observed normal structure in the epithelial cell, primary lamellae and secondary lamellae [Figure 2a]. Moderate deformation and loss of secondary lamellae happened during the exposure to dieldrin (0.006 mg/L) after 96-h [Figure 2b]. Severe deformation and loss of secondary lamellae occurred due to the exposure of endosulfan (0.004 mg/L) and heptachlor (0.057 mg/L) after 96-h [Figure 2c,d].
3.5.2. Histopathological Responses in Gill

The histopathological alterations in the gills of African catfish are illustrated in Figure 2a–d after 96-h exposure to endosulfan, dieldrin and heptachlor. Fish in the unexposed group (control) observed normal structure in the epithelial cell, primary lamellae and secondary lamellae [Figure 2a]. Moderate deformation and loss of secondary lamellae happened during the exposure to dieldrin (0.006 mg/L) after 96-h [Figure 2b]. Severe deformation and loss of secondary lamellae occurred due to the exposure of endosulfan (0.004 mg/L) and heptachlor (0.057 mg/L) after 96-h [Figure 2c, d].

3.5.3. Histopathological Responses in Muscle

During the 96-h exposure to endosulfan, dieldrin and heptachlor, the changes in the muscle tissues of African catfish are presented in Figure 3a–d. Fishes in the control group (unexposed) revealed typical structures of muscle tissue including normal epidermis, myotomes and septum [Figure 3a] whereas seriously disintegrated myotomes, disintegrated epidermis and noticeable lesions are found in the muscle tissues after 96-h exposure of dieldrin (0.006 mg/L) [Figure 3b] and heptachlor (0.057 mg/L) [Figure 3d]. Moderate disintegration of myotomes and epidermis are observed in the muscle tissues after 96-h exposure of endosulfan (0.004 mg/L) [Figure 3c].
Figure 2. Histopathological alterations in the gill tissue of *Clarias gariepinus* at 96-h exposure of endosulfan, dieldrin and heptachlor compared to control (unexposed) using H and E 40× magnification. (a) Gill (Control): EPC: Epithelial cell, SL: Secondary lamellae, PL: Primary lamellae. (b) Gill (Exposed to 0.006 mg/L dieldrin): DSL: Deformed secondary lamellae. (c) Gill (Exposed to 0.004 mg/L endosulfan): DSL: Deformed secondary lamellae and Figure 2d. Gill (Exposed to 0.057 mg/L heptachlor): DSL: Deformed secondary lamellae.

Figure 3. Histopathological alterations in the muscle tissue of *Clarias gariepinus* at 96-h exposure of endosulfan, dieldrin and heptachlor compared to control (unexposed) using H and E 40× magnification. (a) Muscle (Control): Epd: Epidermis, Myt: Myotomes, Spm: Septum. (b) Muscle (Exposed to 0.006 mg/L dieldrin): Dmt: Disintegrated myotomes, Lsn: Lesions. (c) Muscle (Exposed to 0.004 mg/L endosulfan): Dmt: Disintegrated myotomes, Dte: Disintegrated epidermis. (d) Muscle (Exposed to 0.057 mg/L heptachlor): Dmt: Disintegrated myotomes, Dte: Disintegrated epidermis.
4. Discussion
4.1. Median Lethal Concentration (LC\textsubscript{50}) for 96-h Exposure to Endosulfan, Dieldrin and Heptachlor

The 96-h LC\textsubscript{50} value (0.004 mg/L) of endosulfan for African catfish determined in the present study is less than the values of 0.035 mg/L for Anabas testudineus [25], 0.024 mg/L for Channa punctatus [40], 0.01 to 0.013 mg/L for Nile tilapia, Oreochromis niloticus [31,41], 0.0078 mg/L for Silver perch, Bidyanus bidyanus [42] and 0.041 mg/L for European eel, Anguilla anguilla [43].

In contrast, the 96-h LC\textsubscript{50} value (0.004 mg/L) of endosulfan for African catfish determined in the present study is higher than the LC\textsubscript{50} for Rainbow trout, Oncorhynchus mykiss (0.0016 to 0.0018 mg/L) [20,42], Asian swamp eel, Monopterus albus (0.0004 mg/L) [24], Perciformes, Cichlasoma dimerus (0.0026 mg/L) [44], Tilapia, Oreochromis mossambicus (0.0036 mg/L) [45], Tilapia fingerling, Oreochromis mossambicus (0.0014 mg/L) [46] and European carp, Cyprinus carpio (0.002 mg/L) [47].

It was observed that the 96-h LC\textsubscript{50} values for both dieldrin and heptachlor from the present study are consistent with the values from the studies presented in Table 11.

Table 11. Comparison of LC\textsubscript{50} for the toxicity of dieldrin and heptachlor pesticides exposed to different fish species.

| Contaminant | Pesticide | Fish Species | Life Stage | Test Type | Test Duration | LC\textsubscript{50} (mg/L) | SOURCE |
|-------------|-----------|--------------|------------|-----------|---------------|----------------|--------|
| Dieldrin    | African catfish, Claris gariepinus Bluegill, Lepomis macrochirus Turbot, Psettta maxima Embryo-larvae | Juvenile | Static | 96-h | 0.006 | Present study |
|             | Bluegill, Striped bass, Morone saxatilis Striped mullet, Mugil cephalus Northern puffer, Sphoeroides maculatus Threespine stickleback, Gasterosteus aculeatus Bluegill, Lepomis macrochirus Rainbow trout, Oncorhynchus mykiss Common goby, Pomatoschistus microps Plaice, Pleuronectes platessa | Adult | Semi-static | 24-h | 0.0055 | [49] |
|             | Fathead minnow, Pimephales promelas | Fingerling | Static | 96-h | 0.094 | [14] |
|             | Guppy, Pocilia reticulata | Fingerling | Static | 96-h | 0.11 | [14] |
|             | Goldfish, Carassius auratus Black bullhead, Ictalurus melas | Fingerling | Static | 96-h | 0.23 | [14] |
|             | Bluegill sunfish, Lepomis macrochirus Rainbow trout, Oncorhynchus mykiss Channel catfish, Ictalurus punctatus Reader sunfish, Lepomis microlophus Largemouth bass, Micropterus salmoides | Fingerling | Static | 96-h | 0.017 | [50] |

It is worthy of mention that LC\textsubscript{50} is dependent on diverse factors such as the method of acute toxicity test, purity percentage of the exposure contaminants and the size and health status of fish used in the toxicity tests. For instance, [20,42] reported LC\textsubscript{50} values of 0.0016 and 0.0018 mg/L for Rainbow trout using static and semi-static toxicity test, respectively. Contrarily, [45,46] obtained LC\textsubscript{50} values of 0.0036 and 0.0014 mg/L for Tilapia of unequal sizes; i.e., 46.78 g fish and fingerlings, respectively. Environmental factors such as temperature, pH, alkalinity and turbidity were also reported to correlate with
endosulfan toxicity [51]. For example, a pH of less than 5 would increase hydrolyzation of endosulfan to endosulfan sulphate, which is relatively more toxic [52]. LC$_{50}$ of Rainbow trout depend on temperature and water quality parameters such as pH, alkalinity and hardness in addition to the fish sizes [20]. Moreover, organisms are usually exposed to several stressors at the same time that may interact with each other and lead to the different synergistic effects [53,54].

4.2. Behavioural Abnormalities of Test Fish during the Exposure to Pesticides

In our study, we observed several behavioral abnormalities of African catfish in response to acute exposure to endosulfan, dieldrin and heptachlor. The reported behavioral alterations such as hyperactivity, increased erratic swimming, excessive mucus secretion, loss of equilibrium, increase jerky movement, gasping and decreased fin movement are similar to patterns observed in zebrafish, Danio rerio [35] following exposure to endosulfan; in Clarias gariepinus [33] for the treatment of chloropyrifos; in Channa punctatus [56,57] with the exposure of cypermethrin; in Cyprinus carpio [58] by profenofos; in Clarias gariepinus [59] for malathion treatment as well as in Rainbow trout, Oncorhynchus mykiss [60] due to the exposure of carbosulfan pesticide. Behavioural abnormalities reduce the ability of fish to adequately respond to several environmental stimuli, which may for instance lead to lower foraging success and a higher susceptibility to predation [34,36].

4.3. Histopathological Responses of Liver, Gill and Muscle Tissues of Clarias gariepinus Due to the 96-h Exposure to Endosulfan, Dieldrin and Heptachlor

In our experiment, histology of liver of Clarias gariepinus exposed to endosulfan (0.004 mg/L), dieldrin (0.006 mg/L) and heptachlor (0.057 mg/L) revealed pyknotic nuclei with congestion of RBCs (red blood cells) in HPV (hepatic portal vein) of hepatocytes, hepatocytes fusion, vacuolization in hepatocytes, melano-macrophages canter and dis-integration of hepatocytes cell membrane with oozing of cytoplasmic content. Our findings are comparable with the histological alterations of liver in Puntius conchonius [61], Oncorhynchus mykiss [60], Cichlasoma dimerus [44], Oreochromis mossambicus [45], Heteropneustes fossilis [62] exposed to endosulfan and dieldrin such as organochlorine pesticides. The present study showed that there is a strong link between liver damage and toxicants. Histological anomalies observed in fish liver tissue after acute exposure of organochlorine pesticides can cause functional disabilities resulting in malfunctioning of various organ systems [60].

In our study, histopathology of gill of C. gariepinus exposed to endosulfan (0.004 mg/L), dieldrin (0.006 mg/L) and heptachlor (0.057 mg/L) presented thickening of primary lamellae epithelium, shorting of secondary lamellae, epithelial hyperplasia and lamellar fusion (fusion of secondary lamellae) with thickening of primary lamellar epithelium along with collapsed secondary lamellae. To validate our findings, similar histological alterations are also found in the gill of Chanos chanos [63], Oncorhynchus mykiss [60], Cichlasoma dimerus [44], Oreochromis mossambicus [64], Salmo salar [65], Channa punctatus [66] as well as Hypessobrycon bifasciatus and Danio rerio [67] after acute exposure to endosulfan, as with other organochlorine pesticides. Exposure of fish to pollutants such as pesticides collapses gills which may disrupt gas exchange efficiency resulting in respiratory disorders, osmoregulatory dysfunction and ion-regulation imbalance to force the excretion of nitrogenous wastage products [68].

The histopathological transformations in the muscle of African catfish, Clarias gariepinus are in agreement with the histological alterations in zebrafish, Danio rerio [69], Hoplias alabaricus [70], Mugil capito [71] as well as redbelly Tilapia, Tilapia zillii and Common sole, Solea vulgaris [72].

The liver plays vital physiological functions such as detoxification of xenobiotics, synthesis of components of the blood, glycogen storage along with release of glucose in the blood [73]. Fish gills provide essential functions such as gas exchange, ion transportation, nitrogenous wastage excretion as well as uptake and excretion of particular xenobiotics [74]. In spite of having detoxifying capacity, the balancing system of liver
may be collapsed showing its structural disruption due to the enhanced concentration of hazardous compounds [75].

Based on the LC$_{50}$ values obtained from this study, it is clear that *Clarias gariepinus* is highly susceptible to the effect of endosulfan, dieldrin and heptachlor. Therefore, these pesticides could cause severe behavioural abnormalities and serious structural changes of the vital organs, i.e., liver and gill of the fish which threaten their population in the rice field ecosystem.

5. Conclusions

This work investigates the acute toxicity of endosulfan, dieldrin and heptachlor to African catfish *Clarias gariepinus* through dose-response relationship, including also behavioral abnormalities and histopathological alterations of vital organs. The toxicity was observed to increase with the concentration of endosulfan, dieldrin and heptachlor. The behavioral abnormalities were observed with the increasing concentration of endosulfan, dieldrin and heptachlor. Particular structural changes were also observed in the vital organs, i.e., gill, liver as well as muscle tissue with the exposure to median lethal concentration of endosulfan, dieldrin and heptachlor. The histopathological and behavioral changes at sublethal levels could indirectly lead to lower survival and reproduction at the individual level and in this way disrupt the population dynamics of *C. gariepinus*. The fish could also bioconcentrate the pesticides and thus become a hazard to human consumers.

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of ethics protocol of Institutional Animal Care and Use Committee (IACUC), and approved by the Institutional Ethics Committee of Universiti Putra Malaysia (UPM), as accordingly with the ethics approval reference no.: UPM/IACUC/AUP-R019/2021; Dated: 23 July 2021.

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**References**

1. Oruç, E. Özcan Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos. *Pestic. Biochem. Physiol.* 2010, 96, 160–166. [CrossRef]
2. Adhikari, S.; Sarkar, B.; Chatterjee, A.; Mahapatra, C.T.; Ayyappan, S. Effects of cypermethrin and carbofuran hemato-logical parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). *Ecotoxicol. Environ. Saf.* 2004, 58, 220–226. [CrossRef] [PubMed]
3. Rahman, M.Z.; Hossain, Z.; Mollah, M.F.A.; Ahmed, G.U. Effect of diazinum 60 EC on toxicant/xenobiotic and to meet energy required to sustain *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus* Naga. *ICLARM Q.* 2002, 25, 8–12.
4. Turner, K.J.; Sharpe, R.M. Environmental estrogens-present understanding. *Rev. Reprod.* 1997, 2, 69–73. [CrossRef]
5. Waite, D.T.; Grover, R.; Westcott, N.D.; Irvine, D.G.; Kerr, L.A.; Sommerstad, H. Atmospheric deposition of pesticides in a small southern Saskatchewan watershed, Environ. *Toxicol. Chem.* 1995, 14, 1171–1175. [CrossRef]
6. Huang, D.-J.; Wang, S.-Y.; Chen, H.-C. Effects of the endocrine disrupter chemicals chlordane and lindane on the male green neon shrimp (Neocaridina denticulata). Chemosphere 2004, 57, 1621–1627. [CrossRef] [PubMed]

7. Industrial Toxicology Research Centre, ITRC (CSIR). Toxicity Data Handbook; Pesticides-A, TDS-161, Endosulfan; 1989; pp. 292–297.

8. World Health Organization, WHO. Endosulfan: International programme in chemical safety. In Environmental Health Criteria 40; WHO: Geneva, Switzerland, 1984.

9. Howard, P.H.; Sage, G.W.; Jarvis, W.F.; Gray, D.A. Handbook of Environmental Fate and Exposure Data for Organic Chemicals; Lewis Publishers: Chelsea, MI, USA, 1991; pp. 6–13.

10. Shanahan, M. End of the Road for Endosulfan: A Call for Action Against a Dangerous Pesticide; Environmental Justice Foundation Ltd.: London, UK, 2003; Volume 4.

11. Mhadhbi, L.; Beiras, R. Acute Toxicity of Seven Selected Pesticides (Alachlor, Atrazine, Dieldrin, Diuron, Pirimiphos-Methyl, Chlorpyrifos, Diazinon) to the Marine Fish (Turbot, Psetta maxima). Water Air Soil Pollut. 2012, 223, 5917–5930. [CrossRef]

12. Martyniuk, C.J.; Doperalski, N.J.; Kroll, K.J.; Barber, D.S.; Denslow, N.D. Sexually dimorphic transcriptomic responses in the teleostean hypotalamus: A case study with the organochlorine pesticide dieldrin. NeuroToxicology 2013, 34, 105–117. [CrossRef] [PubMed]

13. Martyniuk, C.J.; Feswick, A.; Spade, D.; Kroll, K.J.; Barber, D.S.; Denslow, N.D. Effects of acute dieldrin exposure on neurotransmitters and global gene transcription in largemouth bass (Micropterus salmoides) hypothalamus. NeuroToxicology 2010, 31, 356–366. [CrossRef] [PubMed]

14. Kiellhorn, J.; Schmidt, S.; Mangelsdorf, I. World Health Organization. In Heptachlor: Concise International Chemical Assessment Document; World Health Organization: Geneva, Switzerland, 2006.

15. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Heptachlor/Heptachlor Epoxide; U.S. Department of Health and Human Services: Washington, DC, USA, 2007.

16. Augustijn-Beckers, P.W.M.; Hornsby, A.G.; Wauchope, R.D. The SCS/ARS/CES Pesticide properties database for environmental decision-making. II. Additional Compounds. Rev. Environ. Contam. Toxicol. 1994, 137, 1–82.

17. International Agency for Research on Cancer, IARC. Heptachlor and Heptachlor Epoxide. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans. Available online: http://www.iarc.fr/ishery/countrysector/naso_malaysia/en (accessed on 7 October 2021).

18. Ibrahim, M.S. Chapter 14, Persistent Organic Pollutants in Malaysia. Dev. Environ. Sci. 2007, 7, 629–655. [CrossRef]

19. Mahttiessen, P.; Sheahan, D.; Harrison, R.; Kirby, M.; Rycroft, R.; Turnbull, A.; Volkner, C.; William, R. Use of a Gammarus pulex bioassay to measure the effects of transient carbofuran run off from farmland. Ecotoxicol. Environ. Saf. 1995, 30, 111–119. [CrossRef] [PubMed]

20. Capkin, E.; Altinok, I.; Karahan, S. Water quality and fish size affect toxicity of endosulfan, an organochlorine pesticide, to rainbow trout. Chemosphere 2006, 64, 1793–1800. [CrossRef] [PubMed]

21. Poleksić, V.; Karan, V. Effects of Trifluralin on Carp: Biochemical and Histological Evaluation. Ecotoxicol. Environ. Saf. 1999, 43, 213–221. [CrossRef] [PubMed]

22. Watt, J.; Pierce, G.J.; Boyle, P.R. Guide to the Identification of North Sea Fish Using Prenaxillae and Vertebrae; Trekroner Offset: Denmark, Copenhagen, 1997.

23. Anbu, R.B.; Ramaswamy, M. Adaptive changes in respiratory movements of an air breathing fish, Channa striatus (Bleeker) exposed to carbamate pesticide. J. Ecoliol. 1993, 3, 11–16.

24. Hii, Y.S.; Lee, M.Y.; Chua, T.S. Acute toxicity of organochlorine insecticide endosulfan and its effect on behaviour and some hematological parameters of Asian swamp eel (Monopterus albus, Zuiew). Pestic. Biochem. Physiol. 2007, 89, 46–53. [CrossRef]

25. Nordin, I.; Ibrahim, N.; Ahmad, S.; Hamidin, N.; Dahalan, F.; Shukor, M.A. Endosulfan Toxicity to Anabas testudineus and Histopathological Changes on Vital Organs. E3S Web Conf. 2018, 34, 02055. [CrossRef]

26. Pillay, T.V.R. Aquaculture Principles and Practices; No. Ed. 2; Blackwell Publishing: Hoboken, NJ, USA, 2005.

27. James, M.O.; Stuchal, L.D.; Nyagode, B.A. Glucuronidation and sulfonation, in vitro, of the major endocrine-active metabolites of methoxychlor in the channel catfish, Ictalurus punctatus and induction following treatment with 3-methylcholanthrene. Aquat. Toxicol. Vol. 2008, 86, 227–238. [CrossRef]

28. Erhumwunse, N.O.; Ogbeide, O.S.; Tongo, I.I.; Enuneku, A.A.; Adebayo, P.O. Acute toxicity of glyphosate-based iso-propylamine formulation to juvenile African catfish (Clarias gariepinus). Nig. J. Basic Appl. Sci. 2018, 26, 97–101. [CrossRef]

29. OECD. Fish, Acute Toxicity Testing. In Test Guideline No. 203; OECD: Paris, France, 2019; pp. 1–24.

30. Thoré, E.S.J.; Brendonck, L.; Pinceel, T. Conspecific density and environmental complexity impact behaviour of turquoise killifish (Nothobranchius furzeri). J. Fish Biol. 2016, 97, 1448–1461. [CrossRef]

31. Kenneth, W.; Willem, S. Acute toxicity and lethal body burden of Endosulfan in Tilapia (Oreochromis niloticus (L)). Open Environ. Pollut. Toxicol. J. 2010, 2, 21–26.

32. Uniai, S.H.; Hertika, A.M.S.; Leksono, A.S. Lethal Concentration 50 (LC50—96 hours) Nile Tilapia (Oreochromis niloticus) exposed Cypermethrin-based Pesticide. J. Exp. Life Sci. 2016, 6, 58–62. [CrossRef]

33. Nwani, C.D.; Ivoke, N.; Ugwu, D.O.; Atama, C.; Onyishi, G.C.; Echi, P.C.; Ogbonna, S.A. Investigation on acute toxicity and behavioral changes in a freshwater African catfish, Clarias gariepinus (Burchell, 1822), exposed to organophosphorus pesticide, Termifos. Pak. J. Zool. 2013, 45, 959–965.
34. Halappa, R.; David, M. Behavioural Responses of the Freshwater Fish, *Cyprinus carpio* (Linnaeus) Following Sublethal Exposure to Chlorpyrifos. *Turk. J. Fish. Aquat. Sci.* 2009, 9, 233–238. [CrossRef]  
35. Shivaramaiah, H.M.; Sánchez-Bayo, F.; Al-Rifai, J.; Kennedy, I.R. The Fate of Endosulfan in Water. *J. Environ. Sci. Health Part B* 2005, 40, 711–720. [CrossRef]  
36. Weis, J.S.; Smith, G.; Zhou, T.; Santiago-Bass, C.; Weis, P. Effects of Contaminants on Behaviour: Biochemical Mechanisms and Ecological Consequences. *BiomaScience* 2001, 51, 209–217. [CrossRef]  
37. Little, E.E.; Finger, S.E. Swimming behaviour as an indicator of sub-lethal toxicity in fish. *Env. Toxicol. Chem.* 1990, 9, 13–19. [CrossRef]  
38. Peterson, E.; Buchwalter, D.; Kerby, J.L.; Lefauve, M.K.; Varian-Ramos, C.W.; Swaddle, J.P. Integrative behavioral ecotoxicology: bringing together fields to establish new insight to behavioral ecology, toxicology, and conservation. *Curr. Zool.* 2017, 63, 185–194. [CrossRef]  
39. Bancroft, J.D.; Gamble, M. Hematoxylin and eosin stains. In *Theory and Practice of Histological Techniques*, 6th ed.; Churchill Livingstone: New York, NY, USA, 2007.  
40. Sarma, K.; Pal, A.K.; Baruah, K. Alterations of the ionic composition in different organs of spotted murrel (*Channa punctatus*) exposed to sublethal concentration of endosulfan. *Fish Shellfish. Immunol.* 2009, 27, 105–111. [CrossRef]  
41. Tellez-Bañuelos, M.C.; Santerre, A.; Casas-Solis, J.; Bravo-Cuellar, A.; Zaitseva, G. Oxidative stress in macrophages from spleen of Nile tilapia (*Oreochromis niloticus*) exposed to sublethal concentration of endosulfan. *Fish Shellfish. Immunol.* 2009, 27, 105–111. [CrossRef]  
42. Sunderm, R.I.M.; Thompson, G.B.; Cheng, D.M.H. Toxicity of endosulfan to native and introduced fish in Australia. *Environ. Toxicol. Chem. Int. J.* 1992, 11, 1469–1476. [CrossRef]  
43. Ferrando, M.D.; Sancho, E.; Andreu-Moliner, E. Comparative acute toxicities of selected pesticides to *Anabaena* from different geographical regions of India. *Braz. J. Vet. Res. Anim. Sci.* 2008, 45, 87–94. [CrossRef]  
44. Da Cuña, R.H.; Vázquez, G.R.; Piol, M.N.; Guerrero, N.V.; Maggese, M.C.; Nostro, F.L. Assessment of the acute toxicity of the organochlorine pesticide endosulfan in *Cichlasoma dimerus* (Teleostei, Perciformes). *Ecotoxicol. Environ. Saf.* 2011, 74, 1065–1073. [CrossRef]  
45. Kumar, N.; Prabhu, P.A.J.; Pal, A.K.; Remya, S.; Aklakur, M.; Raman, R.P.; Jadhao, S.B. Anti-oxidative and immuno-hematological status of *Tilapia* (*Oreochromis mossambicus*) during acute toxicity test of endosulfan. *Pestic. Biochem. Phys.* 1999, 65, 45–52. [CrossRef]  
46. Kegley, S.E.; Hill, B.R.; Orme, S.; Choi, A.H. PAN Pesticide Database, Pesticide Action Network, North America. San Francisco, CA. Available online: [http://www.pesticideinfo.org/](http://www.pesticideinfo.org/) (accessed on 7 October 2021).  
47. Salvo, L.M.; Sinhorini, I.L.; Maluccelli, B.E.; Klemz, C.; Sanchez, D.C.O.; Nicarettta, L.; Maluccelli, M.I.C.; Bacila, M.; Assis, H.C.S.D. Effects of endosulfan sublethal concentrations on carp (*Cyprinus carpio*, Linnaeus, 1758): Morphometric, histologic, ultrastructural analyses and cholinesterase activity evaluation. *Braz. J. Vet. Res. Anim. Sci.* 2008, 45, 87–94. [CrossRef]  
48. United States Environmental Protection Agency. Office of Water Regulations, and Standards. In *Criteria & Standards Division*; Ambient Water Quality Criteria for Aldrin/dieldrin; USEPA: Washington, DC, USA, 1980; Volume 80.  
49. Peakall, D.B.B. Dieldrin and other cyclodiene pesticides in wildlife. In *Ambient Water Quality Criteria for Aldrin/dieldrin*; U.S. Department of the Interior, Fish and Wildlife Service: Bailey’s Crossroads, VA, USA, 1986.  
50. Mayer, F.L.; Ellersieck, M.R. *Manual of Acute Toxicity*: Interpretation and Data Base for 410 Chemicals and 66 Species of Freshwater Animals (No. 160); U.S. Department of the Interior, Fish and Wildlife Service: Bailey’s Crossroads, VA, USA, 1986.  
51. Pillai, B.R.; Mahapatra, K.D.; Ponzoni, R.W.; Sahoo, L.; Lalrinlangsanga, P.L.; Nguyen, N.H.; Mohanty, S.; Sahu, S.; Chow, H.L.; Patra, G. Genetic evaluation of a complete diallel cross involving three populations of freshwater prawn (*Macrobrachium rosenbergii*) from different geographical regions of India. *Aquaculture* 2011, 319, 347–354. [CrossRef]  
52. Lee, S.-E.; Kim, J.-S.; Kennedy, I.R.; Park, J.-W.; Kwon, G.-S.; Koh, S.-C.; Kim, J.-E. Biotransformation of an Organochlorine Insecticide, Endosulfan, by *Anabaena* Species. *J. Agric. Food Chem.* 2003, 51, 1336–1340. [CrossRef]  
53. Thoré, E.S.; Van Hooreweghe, F.; Philippe, C.; Brendonck, L.; Pinceel, T. Generation-specific and interactive effects of pesticide and antidepressant exposure in a fish model call for multi-stressor and multigenerational testing. *Aquat. Toxicol.* 2021, 232, 105743. [CrossRef]  
54. Philippe, C.; Gregoire, A.F.; Thoré, E.; De Boeck, G.; Brendonck, L.; Pinceel, T. Protocol for Acute and Chronic Ecotoxicity Testing of the Turquoise Killifish *Nothobranchius furzeri*. *J. Vis. Exp.* 2018, 134, e57308. [CrossRef]  
55. Shao, B.; Zhu, L.; Dong, M.; Wang, J.; Xie, H.; Zhang, Q.; Du, Z.; Zhu, S. DNA damage and oxidative stress induced by endosulfan exposure in Zebra fish *Danio rerio*. *Ecotoxicology* 2012, 21, 1533–1540. [CrossRef]  
56. Ansari, R.A.; Rahman, S.; Kaur, M.; Anjum, S.; Raisuddin, S. In vivo cytogenetic and oxidative-stress-inducing effects of cypermethrin in freshwater fish, *Channa punctata* Bloch. *Ecotoxicol. Environ. Saf.* 2011, 74, 150–156. [CrossRef]  
57. Pandey, A.K.; Nagpure, N.S.; Trivedi, S.P.; Kumar, R.; Kushwaha, B.; Lakra, W.S. Investigation on acute toxicity and behavioral changes in *Channa punctata* (Bloch) due to organophosphate pesticide profenofos. *Drug Chem. Toxicol.* 2011, 34, 424–428. [CrossRef]  
58. Ahmad, Z. Acute and hematological changes in common carp (*Cyprinus carpio*) caused by diazinon exposure. *Afri. J. Biotech.* 2011, 10, 13852–13859.
59. Ahmad, Z. Toxicity bioassay and effects of sublethal exposure of malathion on biochemical composition and hematological parameters of Clarias gariepinus. Afr. J. Biotech. 2012, 11, 8578–8585.

60. Altinok, I.; Capkin, E. Histopathology of Rainbow Trout Exposed to Sublethal Concentrations of Methiocarb or Endosulfan. Toxicol. Pathol. 2007, 35, 405–410. [CrossRef] [PubMed]

61. Gill, T.; Pande, J.; Tewari, H. Hepatopathtotoxicity of three pesticides in a freshwater fish, Puntius conchoniusham. J. Environ. Sci. Health Part A Environ. Sci. Eng. Toxicol. 1990, 25, 653–663. [CrossRef]

62. Maurya, P.K.; Malik, D.S.; Yadav, K.K.; Gupta, N.; Kumar, S. Haematological and histological changes in fish, Heteropneustes fossilis exposed to pesticides from industrial waste water. Human Ecol. Risk Assess. Int. J. 2019, 25. [CrossRef]

63. Kumar, N.; Ambasankar, K.; Krishnani, K.; Gupta, S.; Bhushan, S.; Minhas, P. Acute toxicity, biochemical and histopathological responses of endosulfan in Chanos chanos. Ecotoxicol. Environ. Saf. 2016, 131, 79–88. [CrossRef]

64. Kumar, N.; Gupta, S.; Chandan, N.K.; Aklakur, M.; Pal, A.K.; Jadhao, S.B. Lipotropes protect against patho-gen-aggravated stress and mortality in low dose pesticide-exposed fish. PLoS ONE 2014, 9, e93499.

65. Glover, C.N.; Petri, D.; Tollefsen, K.-E.; Jørum, N.; Handy, R.D.; Berntssen, M.H. Assessing the sensitivity of Atlantic salmon (Salmo salar) to dietary endosulfan exposure using tissue biochemistry and histology. Aquat. Toxicol. 2007, 84, 346–355. [CrossRef]

66. Sarma, K.; Pal, A.K.; Sahu, N.P.; Dalvi, R.S.; Chatterjee, N.; Mukherjee, S.C.; Baruah, K. Acute and chronic effects of endosulfan on the haemato-immunological and histopathological responses of a threatened freshwater fish, spotted murrel, Channa punctatus. Fish Physiol. Biochem. 2011, 38, 499–509. [CrossRef]

67. Jonsson, C.M.; Toledo, M.C.F. Acute toxicity of endosulfan to the fish Hypessobrycon bifasciatus and Brachydanio rerio. Arch. Environ. Contam. Toxicol. 1993, 24, 151–155. [CrossRef]

68. Banaee, M. Physiological dysfunction in fish after insecticides exposure. In Insecticides-Development of Safer and More Effective Technologies; Intech Open: London, UK, 2013; pp. 103–142.

69. Bhuvaneshwari, R.; Padmanaban, K.; Rajendran, R.B. Histopathological Alterations in Muscle, Liver and Gill Tissues of Zebra Fish Danio rerio due to Environmentally Relevant Concentrations of Organochlorine Pesticides (OCPs) and Heavy Metals. Int. J. Environ. Res. 2015, 9, 1365–1372.

70. Miranda, A.; Roche, H.; Randi, M.; Menezes, M.; Ribeiro, C.O. Bioaccumulation of chlorinated pesticides and PCBs in the tropical freshwater fish Hoplias malabaricus: Histopathological, physiological, and immunological findings. Environ. Int. 2008, 34, 939–949. [CrossRef] [PubMed]

71. Mohamed, F.A.S.; Gad, N.S. Environmental pollution-induced biochemical changes in tissues of Tilapia zillii, Solea vulgaris and Mugil capito from Lake Qarun, Egypt. Global Vet. 2008, 2, 327–336.

72. Mohamed, F.A. S Histopathological studies on Tilapia zillii and Solea vulgaris from Lake Qarun, Egypt. World J. Fish Mar. Sci. 2009, 1, 29–39.

73. Brusle, J.; i Anadon, G.G. The structure and function of fish liver. In Fish morphology; Routledge: Oxfordshire, UK, 2017; pp. 77–93.

74. Evans, D.H.; Piermarini, P.M.; Choe, K.P. The Multifunctional Fish Gill: Dominant Site of Gas Exchange, Osmoregulation, Acid-Base Regulation, and Excretion of Nitrogenous Waste. Physiol. Rev. 2005, 85, 97–177. [CrossRef]

75. Ortiz, J.B.; de Canales, M.L.G.; Sarasquete, C. Histopathological changes induced by lindane (g-HCH) in various organs of fishes. Sci. Mar. 2003, 67, 53–61.