

Edibility of Selected Freshwater Fish from the Rietvlei Dam

Report to the
WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

BACKGROUND

Eutrophication is a widespread problem in most of South African water resources and usually a result of excess nutrients that stimulate plant growth (algae, periphyton attached algae, and nuisance plant weeds). Plant growth causes a decline in dissolved oxygen as a result of decomposing plant material causing the death of other organisms. In eutrophic waters the algal production usually exceeds the grazing potential of zooplankton, which in turn is dependant on the availability of algal food and the predation by planktivorous fish. In eutrophic waters there is, however, no shortage of algal food and therefore, the density of zooplankton may be enlarged by reducing the numbers of planktivorous fish.

The manipulation of the food chain in freshwater impoundments has been used as an alternative to restore good quality of water by having more native and advantageous fish species. The restructuring of fish communities, therefore, improve the ecology of a dam. Some experts regard *C. gariepinus* as an undesirable species due to its predatory and bottom-feeding habits. In order to maintain the good ecological functioning of a dam, this species need to be removed and therefore, the fish community will naturally shift towards *Oreochromis mossambicus* (Mozambique tilapia) as the most important species. As part of the food web management/manipulation project nr K5/1643 of DH Consulting, the harvested fish will be sold, which means that the fish will become available for human consumption. Therefore, the risk these fish pose to human health needs to be explored.

RATIONALE

The information generated by this study provides a better understanding of the current presence and extent of contaminants in fish tissues from freshwater environments.

OBJECTIVES AND AIMS

The objective of the project was to determine if harvested *Clarias gariepinus* fish from the Rietvlei Dam (RVD) is safe for human consumption. The aims were:

- To assess the extent to which selected vital target organs of *C. gariepinus* are affected by the chemicals in this impoundment as a possible indicator of potential harmful effects in humans.
- To determine the levels of contaminants in fish tissue samples (muscle and fat) and to compare results with existing international guidelines to determine edibility of the harvested fish.

- To do a human health risk analysis to investigate the possible risk ingested muscle might have on humans consuming these fish.

METHODOLOGY

Twenty (9 male; 11 female) *C. gariepinus* were collected from the RVD, weighed/measured and sexed as male/female/intersex according to the external urogenital papilla. Blood samples were collected for haematocrit (HCT), leucocrit (LCT) and plasma proteins analyses. Each fish was weighed and measured. Fish were killed by a spinal cut and necropsy was performed. The health assessment index (HAI) was used to assess the general health status of each fish in the impoundment. The right pectoral fin from each specimen was removed for age determination.

The spleen, liver and gonad masses were recorded and the somatic indices were calculated. Samples of the liver, gills, testes and ovaries were fixed in preservatives for histological assessment. Available mesenteric fat and muscle samples were collected, wrapped in foil and frozen to do endocrine disrupting chemical (EDC) analyses. Muscle samples were also collected and stored in plastic bags for metal analyses.

The organochlorine pesticides (OCs) and the alkylphenols (APs) were measured by gas chromatography – mass spectrometry (GC-MS), while the metals in the muscle tissue were determined by using inductively coupled plasma-mass spectrometry (ICP-MS). The levels of chemicals in both fish muscle and fish fat were used to perform a risk analyses if the fish was to be ingested by humans (US EPA, 1991).

The human risk analyses included:

- i) Average Daily Dose (ADD) or Exposure Concentration
- ii) Lifetime Average Daily Dose (LADD)
- iii) Carcinogenic Risk
- iv) Non carcinogenic risk – Hazard quotient

RESULTS AND DISCUSSION

The health assessment index (HAI) indicated that both the external features and internal organs were in good physical condition and the condition factor (CF) indicated good health. Two intersex fish were identified according to the macroscopic appearance of gonads. Both fish had male and female characteristics.

These findings indicated that although general fish health is good in RVD, the reproductive health of the catfish was compromised.

p,p'-1,1-dihloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), *p,p'*-1,1-dihloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), *o,p'*-1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), *p,p'*-DDT, gamma (γ)-Lindane, polychlorinated biphenyl (PCB) 153, endosulfan II, metoxychlor, delta (δ)-Lindane, heptachlor, endosulfan I, dieldrin and endrin residues were detected in fat samples of most of the fish. *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT, endosulfan II, δ -Lindane, heptachlor, endosulfan I, dieldrin and endrin residues were detected in some of the muscle samples. Muscle samples contained the metals aluminium (al), arsenic (as), boron (b), chromium (cr), copper (cu), iron (fe), mercury (hg), rubidium (rb), silicon (s), strontium (sr) and zinc (zn).

According to the risk assessment, if the fish is consumed over a long term basis, adverse health effects are expected as a result of dieldrin, aldrin and to a lesser extent heptachlor concentrations found in the fish, as these chemicals are classified as "Probable human carcinogens". Health effects of aldrin and dieldrin in animals include changes in the liver and reduced ability to fight infections. Studies in animals have shown that aldrin and dieldrin may damage sperm. The effects of heptachlor observed in animal studies include damage to the liver, excitability, and decreases in fertility.

GENERAL

This is the first evaluation on the edibility of freshwater fish from impoundments and could serve as model to assess other impoundments of concern.

RECOMMENDATIONS FOR FUTURE RESEARCH

The evaluation of other impoundments where people are dependant on freshwater fish should likewise be evaluated.

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LIST OF ABBREVIATIONS

Al	aluminium
AP	alkylphenols
As	arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
B	boron
BHC	benzene hexachloride
CF	Condition factor
Cr	chromium
CSI	Cardio-somatic index
Cu	copper
DDD	1,1-dihloro-2,2-bis(<i>p</i> -chlorophenyl)ethane
DDE	1,1-dihloro-2,2-bis(<i>p</i> -chlorophenyl)ethylene
DDT	1,1,1-trichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane
EDC	Endocrine disrupting chemical
EDTA	ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
Fe	iron
GC-MS	gas chromatograph – mass spectrometry
GSI	Gonado-Somatic Index
HAI	Health Assessment Index
HCH	Hexachlorocyclohexane
HCT	haematocrit
Hg	mercury
HSI	Hepato-Somatic Index
ICP-MS	inductively coupled plasma- mass spectrometry
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
IRS	Indoor residual spraying
LCT	leucocrit
MCL	maximum contamination level
MMCs	melano-macrophage centers
NBF	neutrally buffered formalin

NRC	National Research Council
OC	organochlorine pesticide
OP	octylphenol
PCB	polychlorinated biphenyl
PFPA	pentafluoropropionic anhydride
<i>p</i> -NP	nonylphenol
Rb	rubidium
RVD	Rietvlei Dam
Si	silicon
Sr	strontium
SSI	Spleno-Somatic Index
UL	upper intake level
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation
WRC	Water Research Commission
Zn	zinc

1 INTRODUCTION AND OBJECTIVES

There is increasing global concern over persistent, bio-accumulative chemicals and their potential for bio-magnification. Aquatic species are particularly vulnerable (Roberts, 2001), and the possible risks include adverse effects on reproductive health, immunity, thyroid function and neurodevelopment. Consumption of fish exposed to these chemicals may possibly lead to comparable or other adverse health effects in humans.

The Rietvlei Dam (RVD) is an urban impoundment and can serve as a model to indicate the impact of pollution on fish and, therefore, provide an indication as to what could be expected from other urban impoundments in the same catchment management area. The RVD is impacted by increasing urbanization, mining, industrial and farming activities. Water and sediment analyses from previous studies (Barnhoorn et al., 2004; Bornman et al., 2007) indicated that the RVD is polluted by both industrial and agrochemicals and that the water had estrogenic activity. These studies also indicated that a significant percentage of male *Clarias gariepinus* specimens had intersex and histological structural changes were found in the gills and liver (Marchand, 2006).

The Water Research Commission (WRC) approved Project 1643 with Dr. WR Harding as project leader: "The potential of food web manipulation for the restoration of eutrophic South African impoundments". The project involves the bulk removal of tolerant fish species from a number of impoundments, including the RVD. These species include *Cyprinus carpio* (common carp), *Chetia flaviventris* (canary kurper), *Clarias gariepinus* (sharp-toothed catfish) and Mozambique tilapia (*Oreochromis mossambicus*). The harvested fish would be sold and, therefore, available for human consumption. There was, however, concern about the assumption that the collected fish would be safe for human consumption due to the fact that endocrine disrupting chemicals were found in the fat of *C. gariepinus* collected from this impoundment (Bornman et al., 2007).

The objective of the present study was to use an indicator fish species to determine if the harvested fish are safe for human consumption. The approach would include chemical analyses of the fat and muscle tissue, and a histology-based fish health assessment of possible health effects in the fish. *C. gariepinus* was selected as the indicator species as this species is endemic to South Africa and present in most freshwater systems. There is also information available from a previous study on health aspects of *C. gariepinus* from the RVD (Bornman et al., 2007), but in that study chemical analyses was only done on fat samples and not on muscle samples.

The aims of the current study were therefore to (1) to assess the extent to which selected vital target organs of *C. gariepinus* are affected by the chemicals in RVD, and (2) to determine the levels of contaminants in fish tissue samples (muscle and fat) and to compare these results with existing international guidelines to determine whether the harvested fish are safe for human consumption by (3) doing a health risk analysis.

2 MATERIAL AND METHODS

2.1 Fish

Twenty live *C. gariepinus* (n = 20: 9 male; 11 female) specimens were collected using gill nets and seine nets (Project 1643, EcoDynamics). Each fish was sexed according to its secondary sexual characteristics (urogenital papilla), and the body mass and length (total and standard) were recorded to use in the calculation of selected somatic indices.

The right pectoral fin from each specimen was removed for age determination. Thin sections of the distal and basal groove of each pectoral spine were placed in ethanol and analysed using a dissection microscope. The annuli surrounding the hollow central cavity of the spine were counted to determine the age of the specimen (Jearld, 1983).

2.2 Health Assessment Index (HAI)

Each specimen was examined externally to identify any abnormalities of the eyes, opercula, skin/scales, fins as well as the presence of external parasites. Blood samples were collected from the caudal artery at the posterior region of the lateral line (Bornman et al., 2007) in ethylenediaminetetraacetic acid (EDTA) vacutainers, stored on ice until the haematocrit (HCT) and leucocrit (LCT) were determined.

Fish were sacrificed by severing the spinal cord anterior to the dorsal fin. The body cavity was opened ventrally and the visceral organs were examined macroscopically to identify any abnormalities and/or internal parasites. The percentage mesenteric fat was also recorded. The body mass and length measurements were used to calculate Fulton's condition factor (CF): $CF = \text{weight (g)} \times 10^5 / \text{length}^3 \text{ (mm)}$ (Carlander, 1969). All of the above mentioned data was subsequently used to calculate the HAI index (Adams et al., 1993).

2.3 Tissue processing for the histological assessment

The gills, liver and gonads were collected for the histological assessment. The spleen, liver and gonad mass were recorded and the somatic indices were calculated: the Hepato-

Somatic Index (HSI), Spleno-Somatic Index (SSI), Gonado-Somatic Index (GSI) and the Cardio-Somatic Index (CSI). The respective testes lengths of each male were also recorded. The selected target organs were sampled at specific, predetermined morphological regions (mid-sections). Samples of the liver and gills were fixed for 48 hours in 10% neutrally buffered formalin (NBF). The testes and ovaries were fixed for 24 hours in Bouins solution.

NBF samples were washed in running tap water for approximately 12 hours and then dehydrated in rising concentrations of ethanol (30; 50 & 70%). Samples fixed in Bouins solution were rinsed in water and then washed in several replacements of 70% ethanol. Samples were further processed and prepared for light microscopy analyses by the pathology laboratory at the Onderstepoort Veterinary Institute of the University of Pretoria. The histology sections were assessed using light microscopy. Digital images were taken using IM50 Image Manager Software (Pixel IT (Pty) Ltd, Sandton, South Africa).

2.4 Target chemical analyses

Mesenteric fat and skeletal muscle samples were collected from each specimen for target chemical analyses. These samples were individually wrapped in aluminium foil (Endocrine disrupting chemical (EDC) analyses) or plastic bags (metal analyses) and stored at -20° Celsius. The samples were analysed by FDA- and Waterlab (PTY) Ltd. Laboratories (Pretoria, South Africa) using Gas Chromatograph – Mass Spectrometry (GC-MS) and Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) (Semi Quantitative) respectively.

EDC analyses included organochlorine pesticides (OCs), and qualitative analyses of alkylphenols (AP). The OCs include: alpha (α)-, beta (β)-, gamma (γ)- (lindane), delta (δ) isomers of hexachlorocyclohexane (HCH), heptachlor, aldrin, dieldrin, heptachlor epoxide, endosulfan I, endosulfan II, endosulfan sulphate, alpha-chlordane, gamma-chlordane, the six 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) isomers (*o,p'*- and *p,p'*-DDT, – 1,1-dihloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) and – 1,1-dihloro-2,2-bis(*p*-chlorophenyl)-ethylene (DDE), endrin, endrin aldehyde, endrin ketone, methoxychlor and polychlorinated biphenyl (PCB) 153. The APs included 4-nonylphenol (*p*-NP) and 4-octylphenol (*p*-OP).

The tissues were analysed for possible OCs and APs according to methods adapted from Bordet et al. (2002) and Croce et al. (2003) respectively. Extractions were done using solid phase C₁₈ cartridges (Waters-Microsep) conditioned with petroleum ether followed by acetone and methanol. The extracts were loaded onto the cartridge and allowed to flow through. The collected eluate was rinsed with acetonitrile and allowed to elute from the cartridges. The samples were evaporated under nitrogen at 35°C and reconstituted into 2 ml

of hexane. A florisisil cartridge was placed on the manifold and after conditioning with 10 ml of hexane, the samples were loaded. The samples were eluted with 10 ml of petroleum ether-diethyl ether (98:2, v/v) and 12 ml of petroleum ether-diethyl ether (85:15, v/v). The two fractions were combined and evaporated under nitrogen to dryness.

The OC residues were analyzed by a GC-MS. The column temperature was increased from 90 to 200°C at a rate of 40°C/min. The temperature of injector and detector was 250 and 200°C, respectively. High purity helium was used as carrier gas at a flow rate of 0.84 ml/min. Samples were injected under splitless injection mode (Villaverde et al., 2008; Bordet et al., 2002). The mass spectra were collected in the electron impact mode at 70eV and the mass-to-charge ratios (m/z) of the ions were used for quantification in selected ion monitoring (SIM) mode. The confirmation of the OCs was done using SIM mode with three selective ions. The quantification was done with one of the selective ions as the detection limit was too low for a full spectrum.

The APs were analyzed by a GC-MS (Agilent 7890 A) equipped with a 5975C mass spectrometer and an Equity 1701 fused silica capillary column (15 m length×0.1 mm i.d.×0.1µm film thickness, Supelco) according to a method by Croce et al. (2003). Data processing was done by the Agilent MSD ChemStation E.01.00.232 software. The mass spectrometer was operated in selected ion monitoring mode (SIM) with pulse splitless injection mode (680kPa). The phenols were analysed as the pentafluoropropionic anhydride (PFPA) derivatives.

2.5 Statistical analyses

The levels of the most prevalent chemicals in both muscle and fat and the age of the specimens were correlated using the Pearson's Correlation Coefficient. The level of significance was set at 5%.

2.6 Risk analysis

This risk assessment provides a first level screening assessment only to indicate whether a potential health risk exists as a result of consumption of fish resulting from exposure to those chemicals examined and the mean concentrations detected. In addition other chemicals such as metals were detected but not included in the estimate. This risk assessment only examines the potential risks based on pesticides detected in fish.

Risks to human health if the fish were to be ingested were calculated based on target chemicals in both fish muscle and fish fat. The human health risk assessment procedure

described by the US EPA (1991) was used. Mean values of the twenty fish were used to best represent the overall risk.

Cancer risks were calculated using the formula:

$$\text{Risk} = e^{-(\text{oral slope factor} * \text{lifetime average daily dose})}$$

Where lifetime average daily dose = average daily dose (exposure period/ lifetime in years)
Exposures were based on the default values described by the exposure assessment handbook. The exposure duration was assumed to be 30 years with a daily consumption averaged to 54g per event.

Non carcinogenic risk – Hazard quotient

For agents that cause non-cancer toxic effects, a Hazard Quotient (H.Q.) is calculated; this compares the expected exposure to the agent to an exposure that is assumed not to be associated with toxic effects. For oral or dermal exposures, the Average Daily Dose (ADD) is compared to a Reference Dose (RfD) and is calculated using the following equation.

$$\text{H.Q.} = \text{Average Daily Dose} / \text{Reference Dose}$$

Reference Doses are a conservative estimate of non-cancer toxic hazards with differences in sensitivity to toxic effects within and between species, and differences in toxic effects between chronic and sub-chronic exposures taken into account. Units are milligrams of contaminant per kilogram of body weight per day.

3 RESULTS

3.1 Health Assessment Index (HAI)

The results of the HAI are presented in Table 1. No abnormalities were identified regarding the eyes, skin, fins or opercula. Macroscopically, the liver, gills, spleen, kidney, heart and hindgut of all the fish were normal (i.e. no discolouration, nodules or growths). However, fatty livers were observed in three specimens. 45% of fish were infested by few to moderate numbers of nematodes inside the visceral cavity. The mean CF for the group was 0.93 ± 0.11 . A value of 1 is considered to be indicative of a healthy specimen. The mean HSI for the group was $1.69\% \pm 0.49$. This falls within range of 1-2% considered to be normal for teleost species. The mean SSI and CSI were $0.18\% \pm 0.11$ and $0.14\% \pm 0.02$ respectively.

The bile colour of all specimens was yellow, which is normal for *C. gariepinus*. The mesenteric fat was estimated to represent <50% of the content of the visceral body cavity in 75% of specimens and >50% in 25% of specimens.

The LCT of 95% of the specimens were within the normal range (<4%) as stipulated by the HAI. The HCT value of 45% of the specimens was outside the normal range (30-45%).

The population mean HAI was calculated for the group (Table 1). The mean value of 23 was below the range calculated for *C. gariepinus* in the study by Marchand (2006) (range: 29-64) indicating less variability from the normal ranges (Adams et al., 1993) in fish assessed in the current study.

According to the macroscopic examination of the urogenital papillae, the sample group had a gender ratio of 9 males and 11 females. However, the macroscopic appearance of the gonads suggested 9 males, 9 females (normal macroscopic appearance) and two possible intersex fish (Figures 1 & 2). In both these specimens, the gonads resembled a possible mixed-sex organ. Both ovarian and testicular tissue was observed as well as the presence of seminal vesicles. In one of these organs, the ovary consisted of a fluid-filled ovarian sac. The fluid drained from this ovary represented 42.6% of the total gonad mass.

Table 1: Health Assessment Index.

Fish #	Fins	Spleen	Hindgut	Kidney	Skin	Liver	Eyes	Opercula	Gills	Pseudobranchs	Parasites	Haematocrit	Leucocrit	HAI
1	0	0	0	0	0	0	0	0	0	0	10	20	30	60
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	30	0	0	0	0	0	0	0	0	0	0	0	30
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	30	0	30
6	0	0	0	0	0	0	0	0	0	0	10	20	0	30
7	0	0	0	0	0	0	0	0	0	0	10	20	0	30
8	0	0	0	0	0	0	0	0	0	0	10	0	0	10
9	0	0	0	0	0	0	0	0	0	0	0	20	0	20
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	10	0	0	10
12	0	0	0	0	0	30	0	0	0	0	10	0	0	40
13	0	0	0	0	0	0	0	0	0	0	10	20	0	30
14	0	30	0	0	0	30	0	0	0	0	0	20	0	80
15	0	0	0	0	0	30	0	0	0	0	0	0	0	30
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	10	10	0	20
19	0	0	0	0	0	0	0	0	0	0	20	20	0	40
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total:	0	60	0	0	0	90	0	0	0	0	100	180	30	

Population HAI 460

Mean: 23

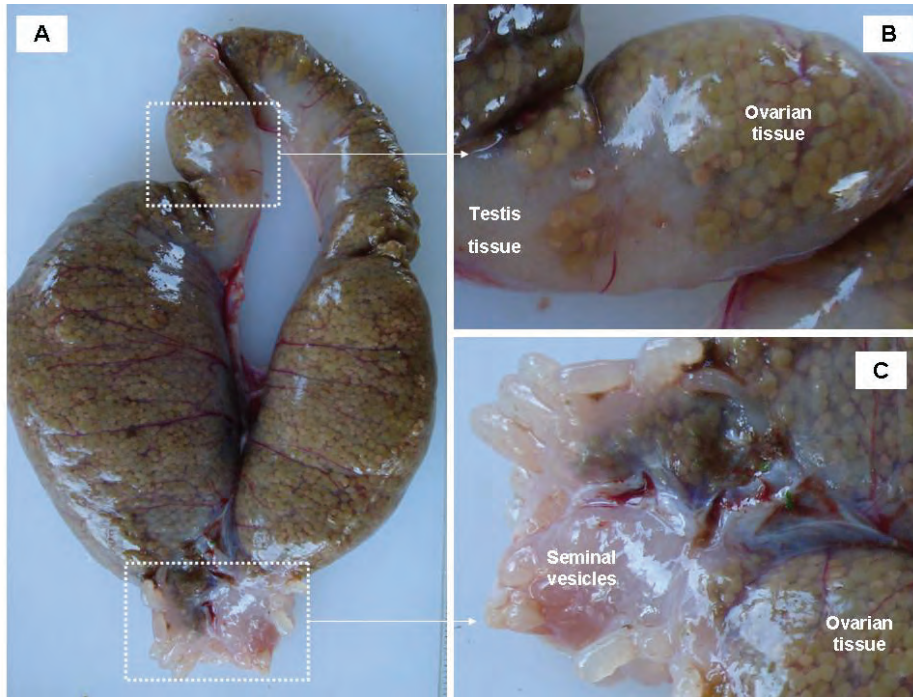


Figure 1: A – Macroscopic view of the mixed sex gonads. B – Anterior region of the organ where both testicular and ovarian tissues are visible. C – Posterior region of the organ showing the seminal vesicles.

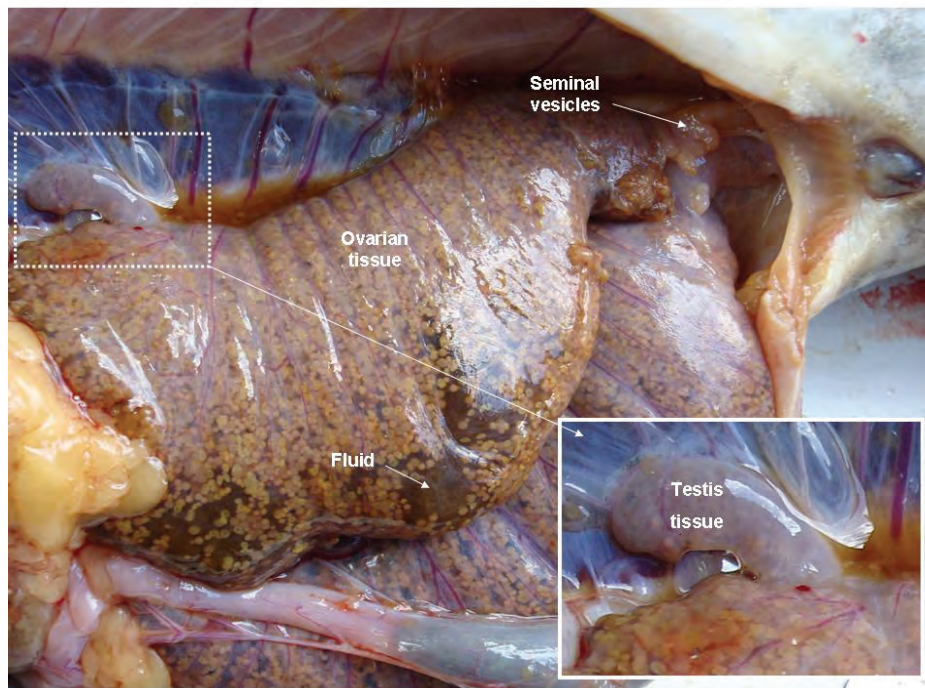


Figure 2: Macroscopic view of the mixed sex gonads. The ovarian sac is filled with fluid. In the posterior region, seminal vesicles are visible and in the dorsal anterior region, testicular tissue is visible.

3.2 Histological assessment

3.2.1 Liver

Thirty-five percent of livers showed structural alterations in the form of cord disarray, 15% of livers had intra-cellular deposits, 70% of livers showed mild to severe steatosis (fatty change) and vacuolated cytoplasm, and 40% had increased amounts of melanomacrophage centers (MMCs) (Figure 3).

3.2.2 Gills

Fifty percent of gills had focal areas of telangiectasia with associated rupture of pillar cells and 25% had focal areas of epithelial lifting of the secondary lamellae (Figure 3). Hyperplasia of the epithelial tissue between secondary lamellae was observed in 15% of specimens while hyperplasia of mucous cells was observed in 65% of specimens.

3.2.3 Gonads

All ovaries (including the two mixed sex organs) were confirmed to be late vitellogenic. In the male group, 33% of the testes were undeveloped, 44% were mid-spermatogenic, and 23% were late spermatogenic. Vacuolated spermatocytes were observed in one male specimen and detachment of the basal membrane of the seminiferous lobules was identified in focal areas in two of the specimens. The histological assessment confirmed that the two mixed sex organs consisted mostly of ovarian tissue and a smaller, separate peripheral region of testicular tissue (including the presence of some spermatozoa) (Figure 3). In none of the fish classified male were any testicular oocytes observed.

To compare the histological results of the current study with a previous study on *C. gariepinus* from the RVD, the results were quantified using an adapted protocol proposed by Bernet et al. (1999). The results are presented in Table 2.

Table 2: Histological index values for *C. gariepinus* from Rietvlei Dam (Mean values are presented).

Sample size (n = 20)	Gill index	Liver index	Testis index	Ovary index	Total organ index
September 2004*	9.5	23.0	10.2	4.0	46.7
January 2005*	8.1	27.6	7.8	2.8	46.3
October 2005*	12.6	22.9	17.8	2.5	55.8
January 2006*	9.1	27.7	7.0	4.5	48.3
October 2007**	8.6	13.0	0.6	2.9	25.1

- * Marchand, 2006
- ** Current study

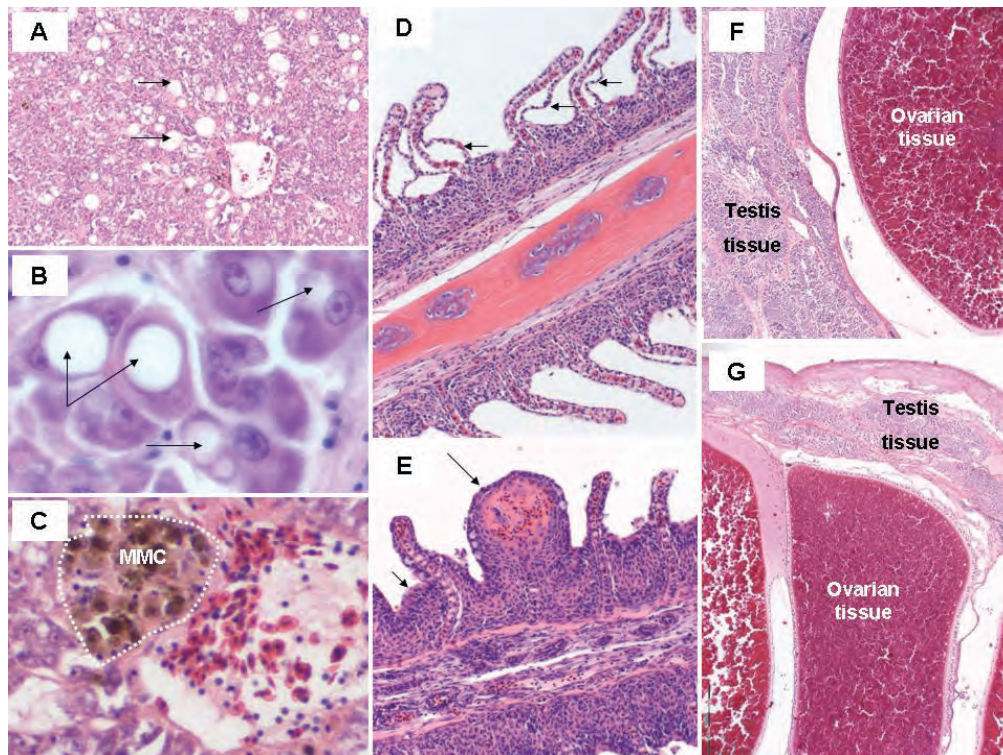


Figure 3: Histological alterations identified in the liver, gill and gonadal tissue. A – Liver sections showing fatty change (arrows) and general disorganization of hepatic structure. B – Detail of lipid vacuoles (arrows) within hepatocytes. C – Melano-macrophage centre (MMC) next to a central vein. D – Intercellular oedema (arrows) of secondary gill lamellae. E – Telangiectasia (long arrow) and hyperplasia (short arrow) of gill epithelium. F & G – Mixed-sex gonad shows both testicular and ovarian tissue within the same organ (H&E).

3.3 The levels of contaminants

3.3.1 OCs in fat and muscle samples

Levels of *o,p'*-DDD, *o,p'*-DDE, α -Lindane, heptachlor, heptachlor epoxide, endosulfan I, dieldrin, endrin, aldrin, β -Lindane were below the detection limit of <50.0 μ g/kg. Table 3 presents the mean (\pm SD) concentrations and the number of samples with levels of selected OCs measured in fat samples.

In muscle samples, *o,p'*-DDD, *o,p'*-DDE, *o,p'*-DDT, α -Lindane, γ -Lindane, PCB153, methoxychlor, β -Lindane were below the detection limit of <50.0 μ g/kg. Table 3 presents the mean (\pm SD) concentrations and the number of samples that contained levels of selected OCs measured in muscle samples.

3.3.2 APs in fat and muscle

The APs could not be determined as the extraction procedure of these compounds from fat and muscle needs to be re-examined and developed to ensure accurate quantitative analyses.

3.3.3 Metal levels in muscle samples

The particular heavy metals detected (mean (\pm SD) concentrations and the number of samples) that contained levels of metals in fish muscle samples are summarized in Table 3. The Silicon (Si) levels were the highest element detected with the maximum and minimum concentrations of 4.38 mg/kg and 0.308 mg/kg respectively. Boron (B) was the second highest metal detected (min: 0.407 mg/kg; max: 1.79 mg/kg) followed by zinc (Zn) (min: 0.191 mg/kg; max: 1.58 mg/kg). Aluminium (Al), arsenic (As), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), rubidium (Rb) and strontium (Sr) were also present in muscle samples. The detection limit for the metal analyses was <0.01 mg/kg.

3.3.4 Statistical analyses

Statistical analyses could only be done on *p,p'*-DDE and showed no correlation between detected in fat or muscle samples and the age of the specimens ($p < 0.05$).

Table 3: Organochlorine pesticides detected (mean \pm SD; $\mu\text{g}/\text{kg}$) in the fat and muscle samples (Detection limit of $50\mu\text{g}/\text{kg}$) and muscle metal concentrations (mean \pm SD; mg/kg ; detection limit of $0.01 \text{ mg}/\text{kg}$) of *C. gariepinus*.

OC detected	Number of fat		Muscle		Number of muscle		Metal detected	Number of muscle	
	Fat samples	samples (n)	samples	samples (n)	samples (n)	samples (n)		Muscle samples	samples (n)
<i>p,p'</i> - DDD	138 \pm 159	4	127 \pm 86	2		19	Al	0.118 \pm 0.083	19
<i>p,p'</i> - DDE	325 \pm 567	18	212 \pm 133	10		19	As	0.328 \pm 0.035	19
<i>o,p'</i> - DDT	61 \pm 9	4				19	B	0.953 \pm 0.551	19
<i>p,p'</i> - DDT	606 \pm 1164	5	198 \pm 77	5		18	Cr	0.024 \pm 0.010	18
Σ DDT	483 \pm 1230	18	177 \pm 254	10		19	Cu	0.020 \pm 0.007	19
γ - Lindane	127	1				19	Fe	0.558 \pm 0.122	19
PCB 153	52	1				4	Hg	0.098 \pm 0.112	4
Endosulfan II	70 \pm 10	6	86 \pm 26	9		19	Rb	0.132 \pm 0.035	19
Metoxychlor	60	1				10	Si	2.627 \pm 1.528	10
δ - Lindane	55 \pm 5	4	55 \pm 7	3		6	Sr	0.012 \pm 0.001	6
Heptachlor			83 \pm 41	5		19	Zn	0.720 \pm 0.566	19
Endosulfan I			58	1					
Dieldrin			77 \pm 36	2					
Endrin			97	1					

3.3.5 Guidelines

Table 4: Available guideline values for OCs in animal tissues.

Toxicant and description	Guideline	Reference
DDT/DDD and DDE		
Fat of meat (cattle, goats, hogs, horses, sheep), fish	5000µg/kg	FDA, 2002
Acceptable daily intake	200µg/kg	WHO, 2002
Hexachlorocyclohexane (HCH)		
Residue Tolerances for γ-HCH		
Cattle, goat, horse, and sheep (fat of meat)	7µg/kg	EPA, 2003n
Hog (fat of meat)	4µg/kg	
Endrin		
Acceptable daily intake	0.2µg/kg	FAO/WHO, 1971
Heptachlor		
Edible fish	300ug/kg	FDA, 2000

3.4 Human health risk assessment

3.4.1 Exposure Scenarios – Ingestion of Fish

Oral ingestion of fish with the specific chemical concentrations used derived from median values of each of the 20 fat and 20 muscle samples taken from the fish from RVD.

An assessment that incorporates other exposures or that does not incorporate all of the exposures described in this analysis will yield different results. This list presents the exposure scenarios evaluated for each contaminated medium considered in this assessment. The dose and concentration estimates in this assessment, refer only to the specific exposures that have been described.

The human risk analyses have been completed using the tables that follow:

Table 5: Chemical concentrations in fish (µg/kg) used in the health risk calculations.

Case #	Chemical	Concentration in fish (µg/kg)
60-57-1	Dieldrin	87
58-89-9	Lindane	55
72-20-8	Endrin	97
76-44-8	Heptachlor	83
50-29-3	DDT	200
309-00-2	Aldrin	80
115-29-7	Endosulfan	58
72-55-9	DDE	200

Table 6: Hazard Identification of the measured chemicals in fish muscle.

Chemical and health effect/s

Dieldrin and Aldrin:

Both Aldrin and Dieldrin are classified as “Probable human carcinogens”. Health effects of aldrin and dieldrin in animals include changes in the liver and reduced ability to fight infections. Studies in animals have shown that aldrin and dieldrin may damage sperm.

Lindane (γ -HCH):

Isomers of hexachlorocyclohexane (HCH) were found to cause liver and kidney effects in animal studies. Reduced ability to fight infection was reported in animals fed γ -HCH, and injury to the ovaries and testes was reported in animals given γ -HCH or β -HCH.

Endrin:

Birth defects such as abnormal bone formation have been seen in some animal studies. There are no human data on birth defects, however evidence in rodents suggests that exposure to high doses of endrin during pregnancy could be a health risk to developing foetuses.

Heptachlor:

Classified as a “Probable human carcinogen”. The effects observed in animal studies include damage to the liver, excitability, and decreases in fertility.

DDT and DDE:

Exposure to DDT or its metabolites during development may change how the reproductive and nervous systems work. This is likely due to its ability to mimic the action of natural hormones. DDT and its isomers are classified as a “Probable human carcinogen”. Studies in animals have shown that oral exposure to DDT can cause liver cancer.

Endosulfan:

The kidney, testes, and possibly the liver are affected by longer-term exposure to endosulphan in laboratory animals. Endosulfan is classified as a “Probable human carcinogen”.

Table 7: General population parameters used for the exposure assessment.

Population: Assuming subsistence fisherman	
Body Weight	70 Kg
Lifetime	70 Years
Exposure Period	10 and 30 years
Event Frequency	350 events per year
Amount ingested	0.05kg per event
Fraction contaminated 100%: this assessments assumes that the only fish ingested is that collected locally.	

To take into account the variation in fish ingestion, a Monte Carlo simulation was carried out to examine the probability distribution of the ingestion volumes based on literature from the USA EPA, Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories Volume 2: Risk Assessment and Fish Consumption Limits Third Edition, (US EPA, 2000). The results of this Monte Carlo are demonstrated below.

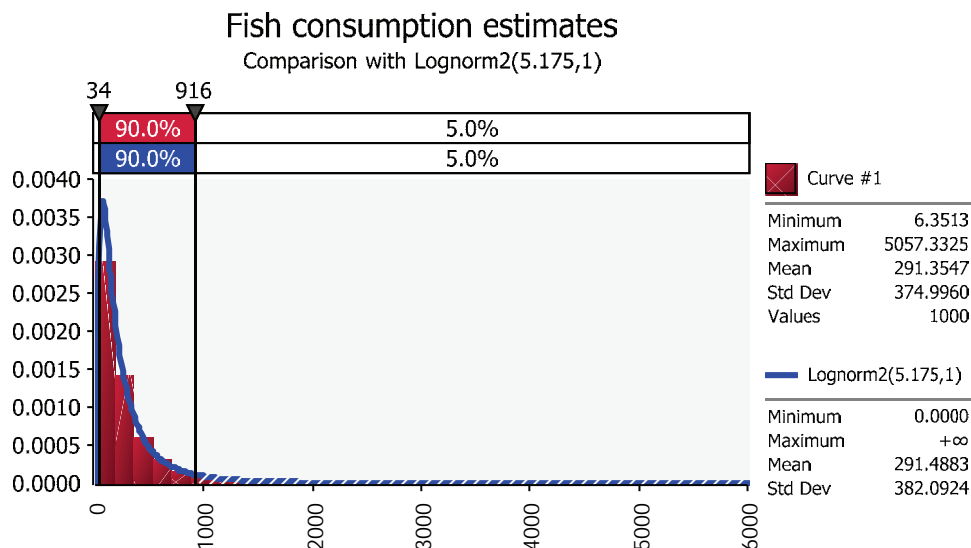


Figure 4: Monte Carlo Probability distribution of fish ingestion amounts.

These ingestions are based on a weekly ingestion amount and this was converted to a mean daily amount ingested.

i) Average Daily Dose (ADD) or Exposure Concentration

When evaluating the risk of chronic non-cancer health effects from oral or dermal exposures, EPA employs the Average Daily Dose (ADD) received during the period of exposure. These are compared to Reference Doses (RfDs).

ADD = Average Daily Dose (during exposure period) in mg/kg body weight per day.

Table 8: Exposure concentrations – Oral average daily dose.

CAS#	Chemical	Oral LADD^ mg/kg/d
60-57-1	Dieldrin	0.000064
58-89-9	Lindane	0.000041
72-20-8	Endrin	0.000072
76-44-8	Heptachlor	0.000061
50-29-3	DDT	0.000148
309-00-2	Aldrin	0.000059
115-29-7	Endosulfan	0.000043
72-55-9	DDE	0.000137

[^]LADD = ADD * (exposure period in years / lifetime in years)

ii) Lifetime Average Daily Dose (LADD)

When evaluating carcinogenic risks from exposures that last less than a lifetime, the ADD or exposure concentration is adjusted to a dose or concentration that would yield an equivalent exposure if exposure continued for the entire lifetime.

Table 9: Lifetime average daily dose calculated based on 10 and 30 years exposure and other assumptions described.

LADD = ADD * (exposure period in years / lifetime in years)			
CAS#	Chemical	Oral LADD mg/kg/d 10 years	Oral LADD mg/kg/d 30 years
50-29-3	DDT	0.000021	0.000063
72-55-9	DDE	0.000021	0.000063
58-89-9	Lindane	0.000006	0.000017
76-44-8	Heptachlor	0.000009	0.000026
72-20-8	Endrin	0.00001	0.000031
309-00-2	Aldrin	0.000008	0.000025
115-29-7	Endosulfan	0.000006	0.000018
60-57-1	Dieldrin	0.00009	0.00028

iii) Carcinogenic Risk

For chemicals that may cause cancer if ingested, risk is calculated as a function of oral Slope Factor and Dose:

$$-(\text{Oral Slope Factor} * \text{Lifetime Average Daily Dose})$$

$$\text{Risk} = 1 - e$$

These estimates represent the risk over background cancer incidence of developing cancer. For example, if the calculated risk is 1 in 1,000,000 (or 1 e-006), this suggests that a person would have a one-in-a-million chance of getting cancer because of the specified chemical exposure, in addition to her/his chance of getting cancer from other causes. It is important to

keep the predicted risks in this perspective as the general risk of developing cancer is 0.25 or a 1 in 4 chance) Reference from the South African National Cancer Association.

Oral Slope of a chemical is described as 1 mg/kilogram of body weight/day). They are generally estimated as the 95th percentile confidence limits using the linearized multistage model, and are conservative estimates of toxic hazard.

The EPA classifies carcinogens according to the strength of evidence of the supporting data with the following used to describe this weight of evidence.

- A = Known human carcinogen.
- B1 = Probable human carcinogen, limited human data.
- B2 = Probable human carcinogen, inadequate or no human data.
- C = Possible human carcinogen.
- D = Not classifiable as human carcinogen.
- E = Evidence that not carcinogenic in humans.

Table 10: Cancer risks based on exposure assumptions described.

Chemical	Weight of evidence	Slope factor(mg/kg/d)	Risk over 10 years exposure	Risk over 30 years exposure
Dieldrin	B2	16	1.00E-04	4.00E-04
Lindane				
Endrin				
Heptachlor	B2	4.5	4.00E-05	1.00E-04
DDT	B2	0.34	7.00E-06	2.00E-05
Aldrin	B2	17	1.00E-04	4.00E-04
Endosulfan				
DDE	B2	0.34	7.00E-06	2.00E-05
Total			3.00E-04	9.40E-04

As a result of the large uncertainties associated with all risk estimates, they should always be interpreted as general indicators, rather than precise estimates. (EPA generally considers risks below 1 in a 100,000 (1e-5) to be acceptable and 1 in 10,000 as unacceptable).

This is represented in the following figure.

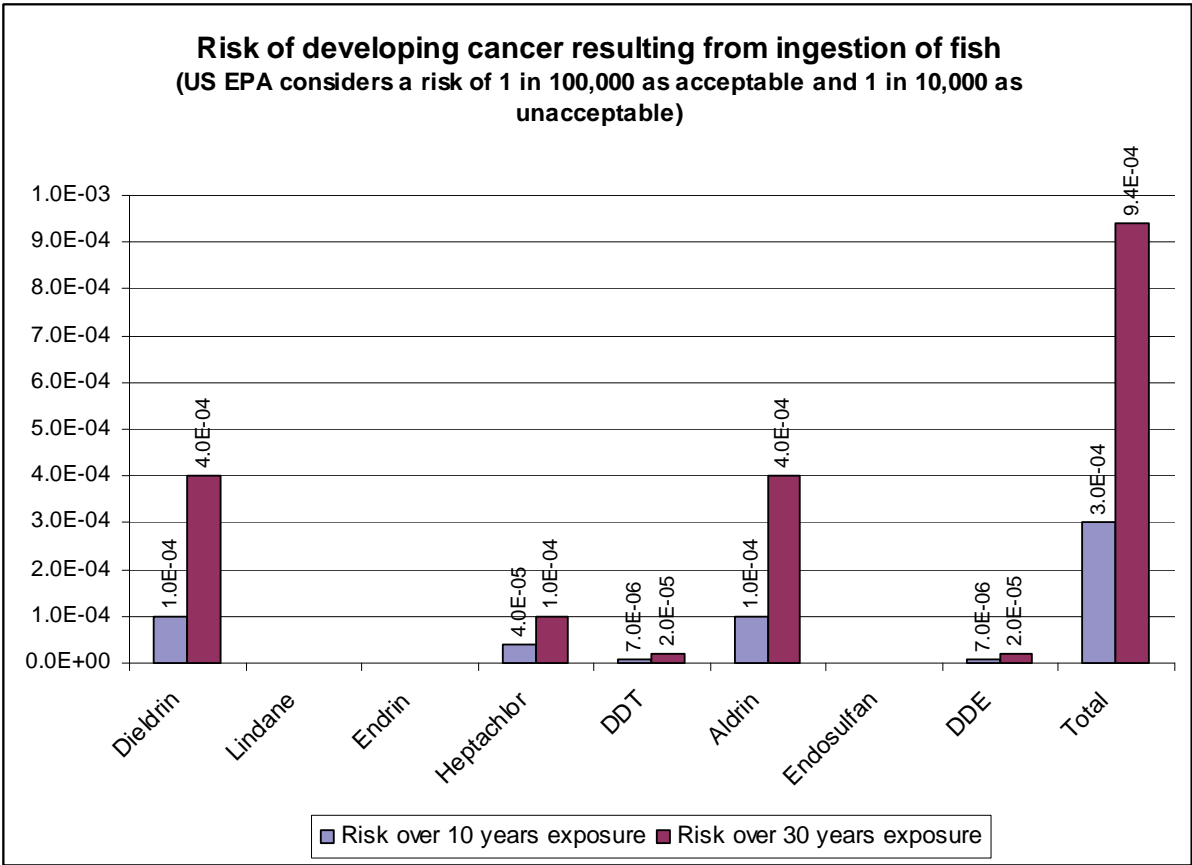


Figure 5: Cancer risks resulting from fish ingestion.

iv) Non-carcinogenic risk – Hazard quotient

For agents that cause non-cancer toxic effects, a Hazard Quotient (H.Q.) is calculated, which compares the expected exposure to the agent to an exposure that is assumed not to be associated with toxic effects. For oral or dermal exposures, the Average Daily Dose (ADD) is compared to a Reference Dose (RfD) and is calculated using the following equation.

$$\text{H.Q.} = \text{Average Daily Dose} / \text{Reference Dose}$$

Reference Doses are a conservative estimate of non-cancer toxic hazards with differences in sensitivity to toxic effects within and between species, and differences in toxic effects between chronic and sub-chronic exposures taken into account. Units are milligrams of contaminant per kilogram of body weight per day.

Table 11: Hazard Quotients due to ingestion of contaminated fish.

Hazard Quotient or Ratio of Average Dose to 'Safe' Daily Dose			
Chemical	RfD (mg/kg/d)	Source	Hazard Index
Dieldrin	0.00005	IRIS(05/30/95)	1.3
Lindane	0.0003	IRIS(05/30/95)	0.14
Endrin	0.0003	IRIS(05/30/95)	0.24
Heptachlor	0.0005	IRIS(05/30/95)	0.13
DDT	0.0005	IRIS(05/30/95)	0.3
Aldrin	0.00003	IRIS(05/30/95)	2
Endosulfan	No RfD		-
DDE	No RfD		-
PCBs	No RfD		-
Total			4.1

These results are shown for all chemicals in the figure below.

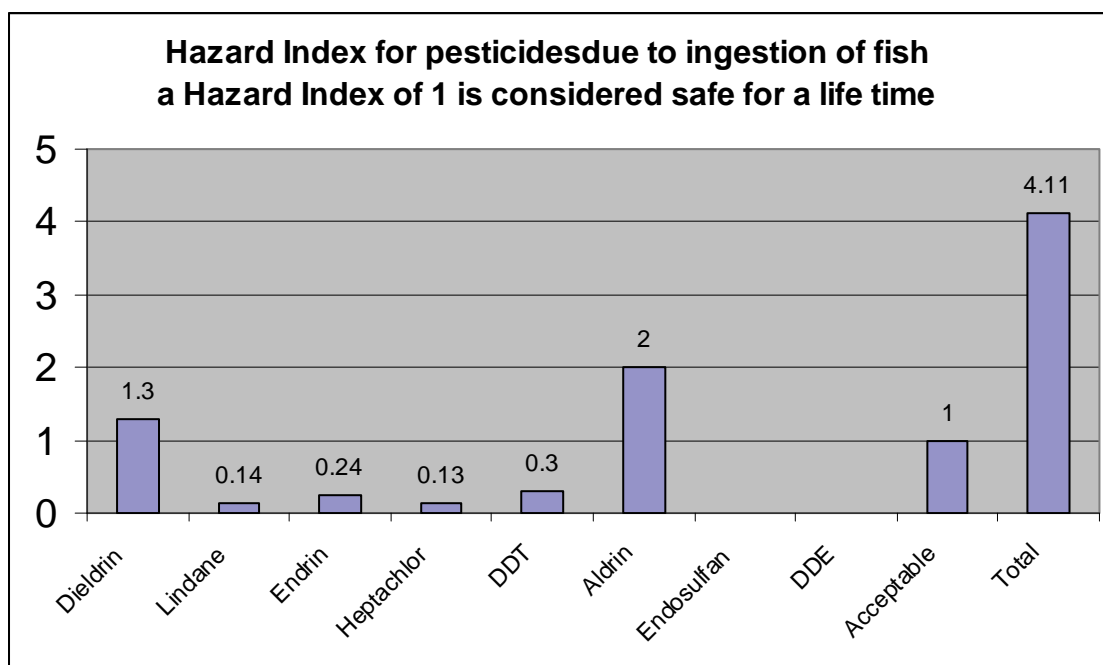


Figure 6: Hazard Index as a result of fish ingestion.

It is generally assumed that non-cancer toxic effects have some threshold. That is, up to some finite level of exposure, physiological defence mechanisms ensure that no toxic effect will occur. Accordingly, hazard assessments for non-carcinogenic effects involve estimating an exposure that is less than this threshold level. This is done by applying "uncertainty factors" to exposures that appear to be near this threshold in laboratory toxicology studies. This yields a Reference Dose (RfD) for oral exposures,

4 DISCUSSION

4.1 Health assessment and histological indices

The majority of parameters measured as part of the HAI were within the normal range as stipulated by Adams et al. (1993). The CF values were also within the normal range. However, a nematode infection was identified in 45% of specimens. The impact of a nematode infection on the health and longevity of fish in nature is generally unknown (Ferris, 1999). It should, however, be noted that the transfer of live fish parasites to humans can occur during consumption of raw fish products.

As stated by Adams et al. (1993), all variables except bile colour and mesenteric fat levels are used in the calculation of the HAI as bile colour can differ depending on the feeding regime of the fish and mesenteric fat deposits can vary widely depending on food availability, feeding regime, fish size, sex, time of year and stress levels.

The histological assessment showed various histological alterations in the selected target organs. The majority of alterations identified are not regarded as toxicant specific and can be the result of exposure to an array of different stressors. However, the organs were found to be in a functional state according to the histological results. The quantitative histological results were compared to a previous study on the health status of *C. gariepinus* from RVD using the same histological assessment protocol (Marchand, 2006) (Table 3). Similar histological index values were calculated for the gill and ovaries in both studies. The Liver indices were higher in the previous study compared to the current study. However, the higher Total index values calculated in the study by Marchand (2006) (80 fish were sampled for this study), is primarily as a result of the higher number of Intersex fish (36.8%, n = 14, Testis index = 10.7) compared to the number of Intersex fish identified in the current study (10%, n = 2, Testis index = 0.6).

From the above it seems that the respiratory and hepatic organs of the fish in RVD are in a functional state. The histological assessment did, however, indicate that the reproductive health of the catfish in the RVD was compromised. This raises concern regarding the potential of similar effects in humans should they be exposed to the cocktail of chemicals present in the RVD.

4.2 Organochlorine pesticides

p,p'-DDE was the most prevalent toxicant found both in the muscle (212µg/kg) and fat (325µg/kg) of *C. gariepinus* while *p,p'*-DDT was the highest in the fat samples. Although

DDT and metabolites are omnipresent anthropogenic environmental contaminants (Willett et al., 1998; Nakata et al., 1998), the high levels in tissues from RVD were not expected as the reserve is about 600km from areas where DDT-application for malaria vector control is still ongoing. The high levels of these chemicals in the fish tissues are most probably the results of contamination of the catchment area through surface runoff, atmospheric deposition, and leaching due to possible past agricultural applications in the catchment area. The RVD might act a sink for these contaminants and resuspension of DDT takes place as a result of mixing during the high flow season and therefore increases the bioavailability and accumulation in fish. The mean total DDT level detected in the fat samples (483µg/kg) was higher than the level recommended for daily intake by the World Health Organisation (WHO) (2002) of 200µg/kg. Humans can get exposed to DDT and its metabolites through ingestion of contaminated food and contaminated feral fish, including the fatty parts.

The presence of the DDT and its metabolites in fat and muscle of wild catfish However, various new reports raise concern on possible health effects of those exposed to DDT as a result of IRS. Associations have been reported between DDT and DDE and type 2 diabetes mellitus (Cox et al., 2007; Rignell-Hydbom et al., 2007), thyroid hormone levels (Meeker et al., 2006; Alvarez-Pedrerol et al., 2008), immune responses in humans (Cooper et al., 2004), *in utero* exposure and delayed neurodevelopment (Eskenazi et al., 2006), higher prevalence of asthma (Sunyer et al., 2005), increased breast cancer risk, particularly if the exposure occurred before the age of 14yrs (Cohn et al., 2007), and also the risk of both seminomatous and non-seminomatous testicular carcinoma associated with DDT and metabolite exposure (McGlynn et al., 2008). Therefore, consumption of contaminated fish may contribute to the total DDT exposure, and is of particular concern in currently DDT-sprayed areas.

HCH is also a ubiquitous contaminant in the environment (Willett et al., 1998). Like OCs, HCH is also banned or restricted in many countries. Lindane, the gamma-HCH (γ -HCH) isomer of HCH or benzene hexachloride (BHC) is used as insecticide on fruit, vegetables, forest crops, animals and -premises. The use of HCH is also restricted in South Africa (Agency for Toxic Substances and Disease Registry (ATSDR), 2005). There are no available δ - and β - Lindane guidelines regarding the safe consumption of contaminated fish.

Endosulfan, a broad spectrum contact insecticide and acaricide, is another pesticide used by many subsistence farmers in African countries (Darko et al., 2008). Endosulfan I and II (α -endosulfan, and β -endosulfan) levels in fish fat and muscle samples suggests that the residues of these chemicals in the water accumulate in resident fish. Although there are no

guidelines available regarding safe levels in fish tissue for human consumption, endosulfan is also listed as an endocrine disruptor (Park et al., 2001) and both the EPA and the ATSDR consider endosulfan to be a potential endocrine disruptor. Numerous *in vitro* studies have documented its potential to disrupt hormones and animal studies have demonstrated its reproductive and developmental toxicity, especially among males (ATSDR, 2000).

Endrin is used as an insecticide and rodenticide and is closely related to aldrin and dieldrin. Of the three, Endrin is very poisonous to humans and acute exposure/toxic dose may lead to excitability and convulsions, and death may follow within 2-12 hours without treatment International Programme on Chemical Safety (IPCS), 1992). Endrin is also toxic to aquatic organisms such as fish, aquatic invertebrates, and phytoplankton (ATSDR, 1996). The EPA's maximum contamination level (MCL) for endrin in drinking water is 0.0002 mg/L. There are also no guidelines regarding safe human consumption of any food type. The muscle sample of one *C. gariepinus* specimen had 97µg/kg of endrin. Fortunately, the reported intake levels of humans are far below the acceptable daily intake of 0.0002 mg/kg body weight established in 1970 (Food and Agriculture Organization of the United Nations (FAO)/WHO, 1971). Three endrin poison episodes have been reported: in Wales (Davies and Lewis, 1956), Saudi Arabia (Weeks, 1967) and a case from the United States (Waller et al., 1992) as a result of endrin contaminated flour.

Methoxychlor is also an insecticide used to protect animals and crops. The EPA has set a MCL of 40µg/kg for drinking water (ATSDR, 2002). According to the EPA, methoxychlor is not a persistent toxicant and is metabolised by soil and sediment microbes. The fat sample of one fish had 59.7µg/kg methoxychlor. However, according to the EPA, most fish and animals transform methoxychlor into other materials that are speedily excreted, preventing build-up thereof in the food chain (ATSDR, 2002). Although humans mostly get exposed to methoxychlor via inhalation or through skin absorption, individuals who eat fish from waters contaminated with methoxychlor, may have above-average intakes of methoxychlor. The EPA has set limits of 1-100 mg/kg on the amount of methoxychlor that is allowed in various agricultural products. Very little research have been conducted on the effects of methoxychlor on humans but the EPA has issued warnings that levels above the MCL of 40 µg/L in drinking water could result in central nervous depression, diarrhoea, damage to liver, kidney, and heart, and – as a result of chronic exposure – growth retardation (ATSDR, 2002).

Another insecticide found in the muscle of *C. gariepinus* from RVD is heptachlor. Trade names include Heptagran®, Basaklor®, Drinox®, Soleptax®, Termide®, Gold Crest H-60®,

and Velsicol 104® (ATSDR, 2007). Fish is known to accumulate levels of heptachlor and humans get exposed through the ingestion of contaminated fish, dairy products, and fatty meats. The limit on food crops is 0.01 mg/kg. The limit in milk is 0.1ppm of milk fat. The limit on edible seafood is 0.3 mg/kg. According to the EPA heptachlor poses no threat to human health (ATSDR, 2007).

4.3 Metals

There are no distinctive guidelines available for permissible metal values in fish tissues regarding safe human consumption. Micronutrients including Cr, Cu, Fe, Si and Zn are regarded as essential elements that help with normal metabolic functions in the body. They are also components of hormones, vitamins and enzymes (Szefer and Nriagu, 2006). Humans usually attain these micronutrients through their daily dietary intake. In order to maintain normal metabolic functions, humans need certain minimum amounts per day, for instance 14-18 mg Fe, 13-16 mg Zn, and 2-2.5 mg Cu. Although these elements are essential, they can be toxic to humans in at high levels and it is necessary to maintain the daily intake below the guidelines values. In fish, metals do accumulate in tissues such as the muscle due to the presence of these metals in the water (Szefer and Nriagu, 2006).

Si, B and As are metalloids which is a group of elements that can not be termed either metal or non-metal due to their general physical and chemical properties. Si is vital to the construction industry as a principal constituent of natural stone, glass, concrete and cement. Silicon has been described as an essential element for plants (Epstein and Bloom, 2005) but have very little benefit as an essential metal in animals and humans. With no known biological function in animals, silicon in excess amounts can cause depression in roughage dry matter, digestibility and formation of urinary calculi in ruminants, and abnormal reproduction in rats (National Research Council (NRC), 2005). It is, however, considered a dietary requirement for humans in terms of bone development. The usual daily intake varies between 20-1200 mg (Greenwood and Earnshaw, 2002). The Si detected in fish from the RVD will not be deleterious to human health.

B is another essential element for animals and plants and although the role thereof is poorly understood, it is considered in the drinking water guidelines of the WHO (2003). Humans usually ingest boron through the consumption of fruits, vegetables and nuts. No health effect related to the element boron has been revealed in humans but data on toxicity to humans focuses on boric acid and borax through inhalation (Stokinger, 1981).

Levels of As can be present in fish and seafood as fish absorb arsenic from the surrounding aquatic environment. Fortunately, this is primarily the moderately harmless organic form of arsenic, although fish that contain significant amounts of inorganic arsenic may be a danger to human health. The US EPA (1999) drinking water criterion for human health protection is 50µg/l, 120 mg As/kg diet, or (in the case of freshwater fish) tissue residues >1.3 mg/kg fresh weight (Eisler, 1988). Freshwater fishes usually contain 0.2-320 mg/kg As (all values are based on dry mass). Most living organisms normally contain measurable concentrations of arsenic, except for marine biota. These are usually less than 1 mg/kg fresh weight. The fish collected for this study had 0.379 mg/kg (maximum) As in the muscle tissue. The legal limit for arsenic in water applied by the WHO (1996) is 10µg/L. In more than one country (Spain and New Zealand) a higher As intake was related to higher fish consumption rate. Too high levels taken in by humans can lead to nausea and vomiting, hemorrhagic diarrhoea and renal failure (Szefer and Nriagu, 2006).

Al is regarded as the most common element in the earth's crust and the second most important mineral. Levels in processed fish consumed in the UK are estimated at 5.5 mg/kg. A lower level of 0.279 mg/kg (maximum) was found in muscle from fish caught in the RVD. There is no guideline for the safe consumption of the edible part of fish. The daily intake of Al ranges in the published literature from 1.53 to 160 mg/person/day (Sorensen et al., 1974). Al has not been regarded as a toxic substance to humans for over a decade and the first illness connected with Al has been discovered in 1974 (Flaten et al., 1996). Excess Al in the body has been implicated as a poisonous driving force in the aetiology of Alzheimer's disease, Guamian amyotrophic lateral sclerosis, and parkinsonism-dementia (Hewitt et al., 1990). Furthermore, carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity have been included (ATSDR, 2008).

There are at least eight essential elements which include Cr and Cu. These metals are also the most important nutrients after calcium and magnesium because of their anti-inflammatory properties. Cr and Cu were found in very low concentrations (Cr 0.02 mg/kg) and (Cu 0.04 mg/kg) in the muscle of *C. gariepinus*. These metal concentrations found in fish tissue did not exceed the Food and Drug Administration standard for edible fish (FDA, 2003).

Rb is present in the earth's crust, and the human body that is easily accumulated in muscles and soft tissues of actinia, worms, molluscs, crustaceans, echinoderms and fish (Gibb, 2007). Rb is not known as a toxic metal but can be dangerous, as it is slightly radioactive. The daily recommended amount of rubidium is 1 to 5 mg. In fish muscle from the RVD Rb

concentrations are below 0.2 mg/kg. There is also no defined recommended daily allowance set for Rb and there are currently no known deficiency or toxicity for Rb (Haas and Levin, 2006).

Fe and Zn are essential elements to most organisms. Fe is however regulated in mammals partly because of the high potential for biological toxicity. Humans consume Fe through their dietary intake which includes fish. The tolerable upper intake level (UL) for adults is stipulated as 45 mg/day and for children under fourteen the level is 40 mg/day (Spanierman, 2007). The consumption of fish is not a common or known route of Zn intake for humans. According to the WHO about half of the population worldwide is at risk for Zn deficiency which can lead to malnutrition and diarrhoea (WHO, 2007). With the low levels of Zn and Fe detected in the muscle (below 1 mg/kg Fe or Zn) the muscle should not hold a risk for Fe or Zn toxicity to humans.

4.4 Human Health risk assessment

Subsistence fishers consume fish as a major staple of their diet. These fishers rely on fish to meet nutritional needs, as an inexpensive food source, and, in some cases, because of their cultural traditions. Subsistence fishers often have higher consumption rates than other fisher groups; however, consumption rates vary considerably among subsistence fishers. Consequently, generalizations should not be made about this fisher group. If studies contained in this section are used to estimate exposure patterns for a subsistence population of concern, care should be taken to match the dietary and population characteristics of the two populations as closely as possible.

Subsistence fishers include a wide variety of people who differ in many respects. Subsistence fishers may consume different types or portions of fish than sport fishers (e.g., organs, whole fish), although individual tastes will vary. Their consumption patterns in this regard may result in greater exposure to contaminants.

According to the risk assessment, if the fish is consumed over a long term basis, adverse health effects are expected. The concentrations of pesticides found in the fish is 4 times that considered to be safe for a lifetime consumption (Hazard index = 4.1). Carcinogenic risks were calculated to be 3 in 10,000 over a 10 year exposure period which increased to almost 1 in 1,000 over a 30 year exposure period. However if consumption of fish is lower than that considered to be a "reasonable" exposure, these risks are considerably reduced. The risk assessment is a first tier or screening exercise and indicates that more information is needed to make an informed decision.

5 CONCLUSIONS

The histological assessment indicated that the reproductive health of *C. gariepinus* in the RVD could be affected by chemicals in the system. This raises concern regarding the potential of similar effects in humans should they be exposed to the cocktail of chemicals present in the RVD.

The mean total DDT level detected in the fat samples (483µg/kg) was higher than the level recommended for daily intake by the World Health Organisation (WHO) (2002) of 200µg/kg but the level in the edible part (muscle) of fish (177µg/kg) were below this value. Humans can get exposed to DDT and its metabolites through ingestion of contaminated food and contaminated feral fish including the fatty parts thereof.

There are no guidelines regarding safe levels in fish tissue for human consumption for HCH and endosulfan, but both the EPA and the ATSDR consider endosulfan to be a potential endocrine disruptor.

There are also no available guidelines for the metals tested for in the muscle and fat of *C.gariepinus*. However, the possible effects of the detected metals are not a certainty as the intake and the availability of the metals were not calculated according to a risk formulation to humans consuming wild fish.

According to the risk assessment, if the fish is consumed over a long term basis, adverse health effects are expected as a result of Dieldrin, Aldrin and to a lesser extent heptachlor concentrations found in the fish. Aldrin, Dieldrin and Heptachlor are all classified as "Probable human carcinogens". Health effects of aldrin and dieldrin in animals include changes in the liver and reduced ability to fight infections. Studies in animals have shown that aldrin and dieldrin may damage sperm. The effects of heptachlor observed in animal studies include damage to the liver, excitability, and decreases in fertility.

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