Freshwater Fish and Human Health

Reference Guide

Ralph Heath, Hein du Preez,
Bettina Genthe & Annemarié Avenant-Oldewage

STEP 1 Selection of scale and depth of survey

STEP 2 Assessment of the water-body catchment

STEP 3 Monitoring system

STEP 4 Field collection

STEP 5 Laboratory sample processing

STEP 6 Analysis of results

STEP 7 Risk assessment

STEP 8 Risk management

STEP 9 Risk communi-cation

STEP 10 Evaluation and review of the programme

TT 213/04

Water Research Commission

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A report to the
Water Research Commission

The publication of this report emanates from a project entitled: *Protocol Manual for the transfer of Methodology required to link Ecosystem Health and Human Health* (WRC Project No. K5/1400B).

For further information refer to:
Freshwater Fish and Human Health
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EXECUTIVE SUMMARY

In South Africa the pollution of freshwater aquatic systems can be linked to point source discharges (waste water treatment works and industrial effluents) and diffuse surface runoff (agricultural, mining and urban). As a result of these anthropogenic activities, innocent people as well as other life forms may be exposed to harmful contaminants, which may be released without adequate consideration of human health and the environmental effects. Studies have shown that when people are exposed to surface water contaminants through contact recreation, drinking water and the consumption of contaminated food their health may be affected.

A review of the published literature revealed that several surveys were undertaken in South Africa to investigate chemical contaminants in freshwater fish. Most of these studies were aimed at contributing to the assessment of the health of the aquatic ecosystem under investigation as they focused on species and tissue differences in contaminant bioaccumulation as well as the spatial and temporal variation in contaminant concentrations. The health risks to humans when consuming contaminated fish are seldom addressed. Furthermore, no standard methodology as for example suggested by the US EPA was followed by the different investigations. This shortcoming limits comparison of data from different studies and prevents accurate determination of risk base fish consumption limits for humans. To address this limitation a generic protocol has been developed that would give guidance in the undertaking of fish contaminant surveys to provide information regarding the possible health risk if the fish are consumed by recreational and subsistence fishermen. As well as to give guidance to surveys investigating the chemical contamination of fish for ecosystem health assessment programmes.

The fundamentals of the protocol are based on catchment information (possible anthropogenic activities that can result in chemical pollution), socio-demographic information of consumers of freshwater fish in the catchment, bioaccumulation potential and health risks of analytes, sound sampling design, risk assessment procedures and performing monitoring at different scales and depth. The methodology identifies ten major steps, namely: (i) selection of scale and depth of survey, (ii) assessment of the waterbody catchment, (iii) monitoring system design, (iv) field collection, (v) laboratory sample processing and analysis, (vi) analysis of and reporting of results, (vii) risk assessment, (viii) risk management, (ix) risk communication and (x) evaluation and review of the programme which are discussed to provide guidance to governmental authorities at national or provincial level and project managers. The basic requirements of each step are highlighted as limited resources (financial, infrastructure and skilled personnel) in South Africa would limit the possibility of undertaking detailed assessments as undertaken by the United States of America Environmental Protection Agency (US EPA). Nevertheless, by applying the proposed protocol, sound comparable assessments, based on risk assessment methodology, can be made regarding the human health risk associated with the consumption of freshwater fish in South Africa.

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GLOSSARY

Acute exposure - Exposure at a relatively high level over a short period of time (minutes to a few days).

Average Daily Dose (ADD) – The amount of the contaminant to which a person is exposed, on average, each day *during* the period of exposure. ADD is generally expressed in milligrams of chemical per kilogram of body mass per day.

Bioaccumulation – The accumulation of a contaminant into an organism or a biological community, resulting either from direct uptake from the water (i.e., by bioconcentration) or from ingestion (i.e., by biomagnification).

Cancer slope factor - The slope of the dose-response curve in the low-dose region used with exposure to calculate the estimated lifetime cancer risk.

Carcinogen - An agent capable of inducing a carcinogenic response.

Chronic exposure – Multiple exposures occurring over an extended period of time, or a significant fraction of the lifetime.

Consumption limits - A daily fish consumption limit, based on health and toxicity data.

Developmental toxicity – Study of adverse effects on the developing organism resulting from exposure prior to conception, during prenatal development, or postnatal up to the time of sexual maturation.

Dose - response - Relationship between the amount of an agent and changes in aspects of the biological system apparently in response to that agent.

Exposure limits - A daily limit on exposure based on health and toxicity data, which the reader may calculate, using the study data provided in this or other sources (mg/kg/d).

Exposure route - The part of the body by which contaminants actually enter the bodies of the exposed population, specifically oral (the route of exposure for contaminants in food, for example), inhalation (exposure route for contaminants in air), and dermal (the most obvious exposure route for contaminants in water during swimming).

Hazard Quotient (H.Q.) – The ratio of the Average Daily Dose (ADD) of a chemical to the Reference Dose (RfD) for that chemical or the ratio of the exposure concentration to the Reference Concentration (RfC). If the H.Q. exceeds one, there is some risk of non-cancer toxic effects for exposure to that specific chemical.

Lowest Observed Adverse Effect Level (LOAEL) – The lowest dose in an appropriate study that *is* associated with an adverse effect on the test organisms.

Modifying factor - A factor used in operationally deriving the RfD from experimental data. It addresses concerns regarding differences in absorption, tolerance to a chemical, or lack of a sensitive endpoint.

Mutagenic – Capable of inducing changes in genetic material (e.g., DNA).

No Observed Adverse Effect Level (NOAEL) - The highest dose in an appropriate study that is *not* associated with an adverse effect on the test organisms.

Pharmacokinetics – The study of the time course of the absorption, distribution, metabolism, and excretion of chemical substances.

Recreational fishers - Non-commercial and non-subsistence fishermen. Synonymous with sport fishermen in this document.

Reference dose (RfD) – Estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (mg/kg/d).

Risk - The probability of injury, disease, or death under specific circumstances.

Risk Level - The maximum acceptable risk level (dimensionless). This is the assigned level of maximum acceptable risks over an individual's lifetime for example RL = 10^{-4} for a level of risk not to exceed one excess case of cancer per 10 000 individual exposed over a 70-year lifetime.

Saxitoxins - A group of carbamate alkaloid neurotoxins which is either non-sulphated, singly sulphate or double sulphated. Saxitoxins from marine dinoflagelates have caused human deaths.

Screening concentration - Concentration of a target analyte in fish tissue that is of potential human health concern and that is used as a standard against which concentrations detected in fish tissue collected from the aquatic environment can be compared to.

Slope factor - The slope of the dose-response curve in the low-dose region used with exposure to calculate the estimated lifetime cancer risk. Most often expressed as risk per milligram of exposure to the toxic chemical per body mass per day. This is usually calculated using the upper 95% confidence limit on the linear term in the linearised multistage model.

Subsistence fishers - Refers in this document to be people who rely on non-commercial fish as a major source of protein.

Teratogenic - Capable of causing physical defects in the developing embryo or fetus.

Toxic hazard –1) The adverse effect or effects that the chemical produces in a species (hazard identification) and 2) the relationship between the amount of chemical and the nature and severity of its adverse effect or in relationship to the frequency of occurrence in a population (dose-effect and dose-response functions, respectively).

Threshold – Dose or exposure below which a significant adverse effect is not expected.

Uncertainty - In risk assessment, uncertainty can be expressed qualitatively or quantitatively. Quantitative descriptions of uncertainty generally take one of two forms: 1) a statement of two alternative estimates (e.g., average case and reasonable maximum) or 2) a probability distribution of potential outcomes. Data is virtually never available to support the second option in a credible fashion.

Uncertainty factors (UF) - One of several, generally 10-fold factors, used in operationally deriving the RfD from experimental data. They are intended to account for (1) the variation in sensitivity among the members of the human population (intraspecies variability); (2) the uncertainty in extrapolating animal data to humans; (3) the uncertainty in extrapolating from data obtained in a study that is of less-than-lifetime exposure to chronic exposure toxicity; (4) the uncertainty in using LOAEL data rather than NOAEL data; and (5) uncertainty generated by data gaps.

A REFERENCE GUIDE FOR DETERMINING THE HUMAN HEALTH RISKS OF CONSUMING FRESHWATER FISH IN SOUTH AFRICA

1.1 INTRODUCTION

Fish forms not only an integral part of aquatic ecosystems, but is an important food source to humans (Zabik *et al*. 1995; US EPA, 1997). Furthermore, most communities that have access to freshwater lakes, reservoirs and rivers practice recreational fishing. However, due to anthropogenic activities the aquatic environment is polluted by chemicals that are accumulated by freshwater fish, and this may pose a health risk to consumers of the contaminated fish (US EPA, 1991a; Bevelhimer, 1995).

A review of the published literature on the occurrence of pollutants in fish from South African freshwater systems revealed that several surveys were undertaken to investigate chemical contaminants in fish. The focus of these investigations was mainly on metal levels (for example the publications by Bezuidenhout *et al*. 1990; du Preez & Steyn, 1992; Grobler, 1994; de Wet *et al*.1994; Grobler *et al*. 1994; Seymore *et al*. 1995, 1996; Claassen 1996; Coetzee, 1996; Schoonbee *et al*. 1996; van Vuren *et al*. 1996; Barnhoorn, 1997; du Preez *et al*. 1997; Kotze, 1997; Kotze *et al.* 1999; Robinson & Avenant-Oldewage, 1997; Nussey, 1998; Heath, 1999; Heath & Claassen, 1999; Nussey *et al.* 1999, 2000; Coetzee *et al* 2002) and biocide concentrations (for example the publications by Bouwman *et al.* 1990; Grobler, 1994; Claassen, 1996; Heath, 1999; Heath & Claassen, 1999) in fish. In general these studies describe the species and tissue differences in contaminant bioaccumulation as well as the spatial and temporal variation in contaminant concentrations. Most of these studies were aimed at contributing to the assessment of the health of the aquatic ecosystem under investigation. The risks to humans when consuming contaminated fish are seldom addressed and only the publications by Claassen (1996), Heath (1999) and Heath & Claassen (1999) used a risk-based approach to assess the possible health risk to humans when consuming fish from selected rivers in South Africa. Furthermore, at present it is not known if any ban has been placed on the consumption of freshwater fish in South Africa. Bans are usually limited to the consumption of shellfish due to the contamination by toxins (Branch & Branch, 1981; WHO, 1999)**.**

Evaluation of the above mentioned South African literature on chemical contaminant levels in freshwater fish clearly shows that no standard methodology as for example suggested by the US EPA (US EPA 1995a,b; 1996; 1997) was followed by the different investigations. Evaluation of published data on contaminant levels in fish from freshwater systems in South Africa clearly indicates that different monitoring programmes have been followed. Although these studies provide contaminant data, many of the data cannot be used in deriving safe consumption levels because:

- The same methodology for reporting of data (e.g. contaminant concentrations as μg/g wet mass or contaminant concentration as μg/g dry mass, data presented as geometric or arithmetic means) was not used.
- Exclusion of critical information, for example lipid concentrations, moisture content and sample size.

- Analyses were performed on non-edible portions of the fish (e.g. gills, gonads, liver tissue, and kidneys).

Similar shortcomings of fish contaminant data were noted by the American National Academy of Science which reviewed 150 reports and publications on seafood contamination in America (NAS, 1991). These shortcomings prevent the accurate determination of human exposure and limit comparison of data from different studies as well as further statistical manipulation and/or risk assessment (US EPA, 1995).

The protocol (Figure 1) developed by Heath (1999) is the first real attempt to standardise and give some guidance on how to perform chemical (pesticides and metals) contaminant bioaccumulation monitoring programme in South Africa. The study by Heath (1999) addresses some of the above-mentioned shortcomings and provides guidance as to which elements constitute a chemical contaminant monitoring programme. However, many of the elements are not discussed in detail and still need further clarification. The issue of the risk to humans when consuming contaminated fish is addressed, but no information regarding the application of the data in the development of fish advisories is given. In contrast, the publication by the US EPA (1995a) gives detailed guidance on methods for sampling and analysing chemical contaminants in fish and shellfish tissue to enhance consistency in data used by the different States of the United States of America when deriving fish and shellfish consumption advisories.

From the preceding it is evident that in South Africa there is a need to standardise the protocol for conducting chemical contaminant surveys using fish and to use this data to protect the health of consumers of freshwater fish. The elements and details discussed in the sections that follow are mainly based on the guidelines given by the US EPA (1995a), the protocol of Heath (1999), other South African studies on freshwater fish.

This report (a reference guide) is one of two that have been developed by the Water Research Commission relating to linking human health and the consumption of freshwater fish. The other report entitled "Freshwater Fish and Human Health - Overview Guide" is available from the Water Research Commission (WRC Report No TT 212/03).

1.2 SPECIFIC COMPONENTS OF THE PROTOCOL

The methodology identifies ten major steps that should be followed in a hierarchal pattern to perform the assessment. Each of these steps is discussed in the sections below.

STEP 1: SELECTION OF SCALE AND SCOPE OF SURVEYS

To optimise resources and to be more cost-effective a monitoring strategy consisting of three levels must be applied (Figure 2 and Figure 3) hierarchical manner:

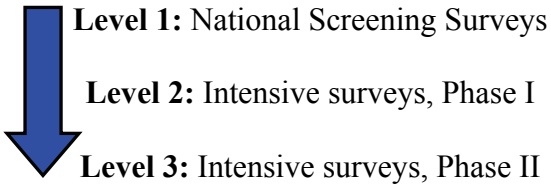
- **Level 1:** *Screening surveys* – A national survey of water-bodies where freshwater fish are captured for commercial, subsistence or recreational purposes and where the water body is of importance in the supply of drinking water or irrigation water as well of ecological importance (for example rivers running through conservation areas). These

surveys thus provide a national state of the water-bodies as well as of where the levels of contaminants in edible fish tissue could cause significant health risks to consumers.

- **Level 2:** *Intensive surveys, Phase 1* – Conduct intensive surveys at sites with potential risks as identified during Level 1 surveys. Therefore determine the magnitude of contamination in edible fish tissue of commonly captured and consumed fish species.
- **Level 3:** *Intensive surveys, Phase II* – Conduct intensive surveys at the sites investigated during Level & 2 surveys in order to determine the level of contamination in specific fish size classes as well as the geographical extent of contamination. A Level 3 survey is therefore more extensive than a Level 2 survey.

The main objective of Level 1 surveys should be to provide a national state of the water-bodies and identify freshwater water-bodies where commercial, recreational or subsistence fishing is practiced and where the levels of chemical contaminants in the edible fish tissue may pose a potential health risk to consumers. The main objective of Level 2 surveys is to determine the magnitude of the contamination in the edible fish tissue of commonly captured fish at the sites as identified during the Level 1 surveys. A Level 3 survey is more detailed and aims to determine the geographical extent of contamination in selected size classes of the most frequently consumed species. These intensive surveys would also be done during impact assessments or during specific case studies.

To be cost effective these levels should be applied in a hierarchical manner namely:



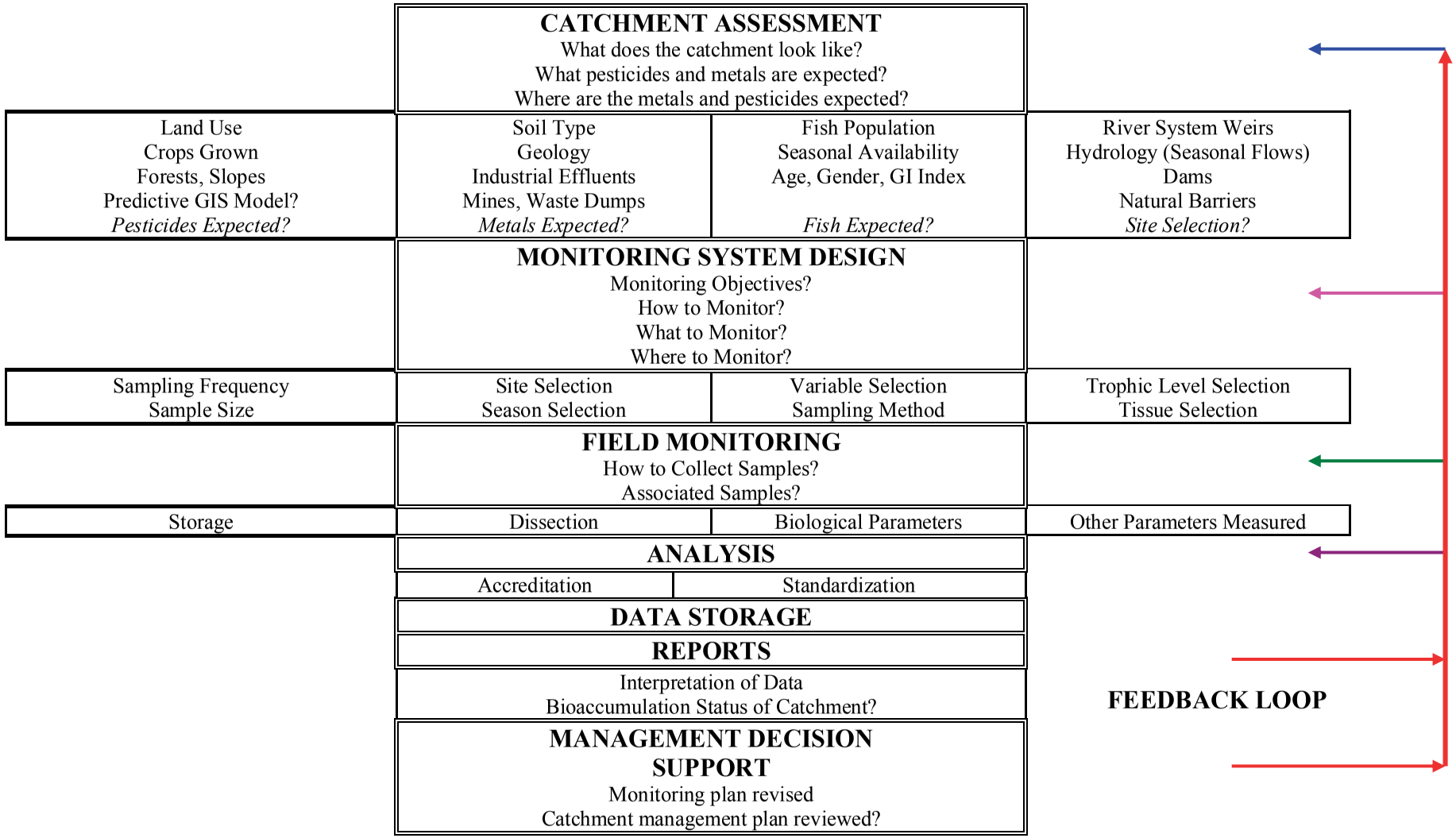


Figure 1. Bioaccumulation protocol and feedback loop proposed by Heath (1999)

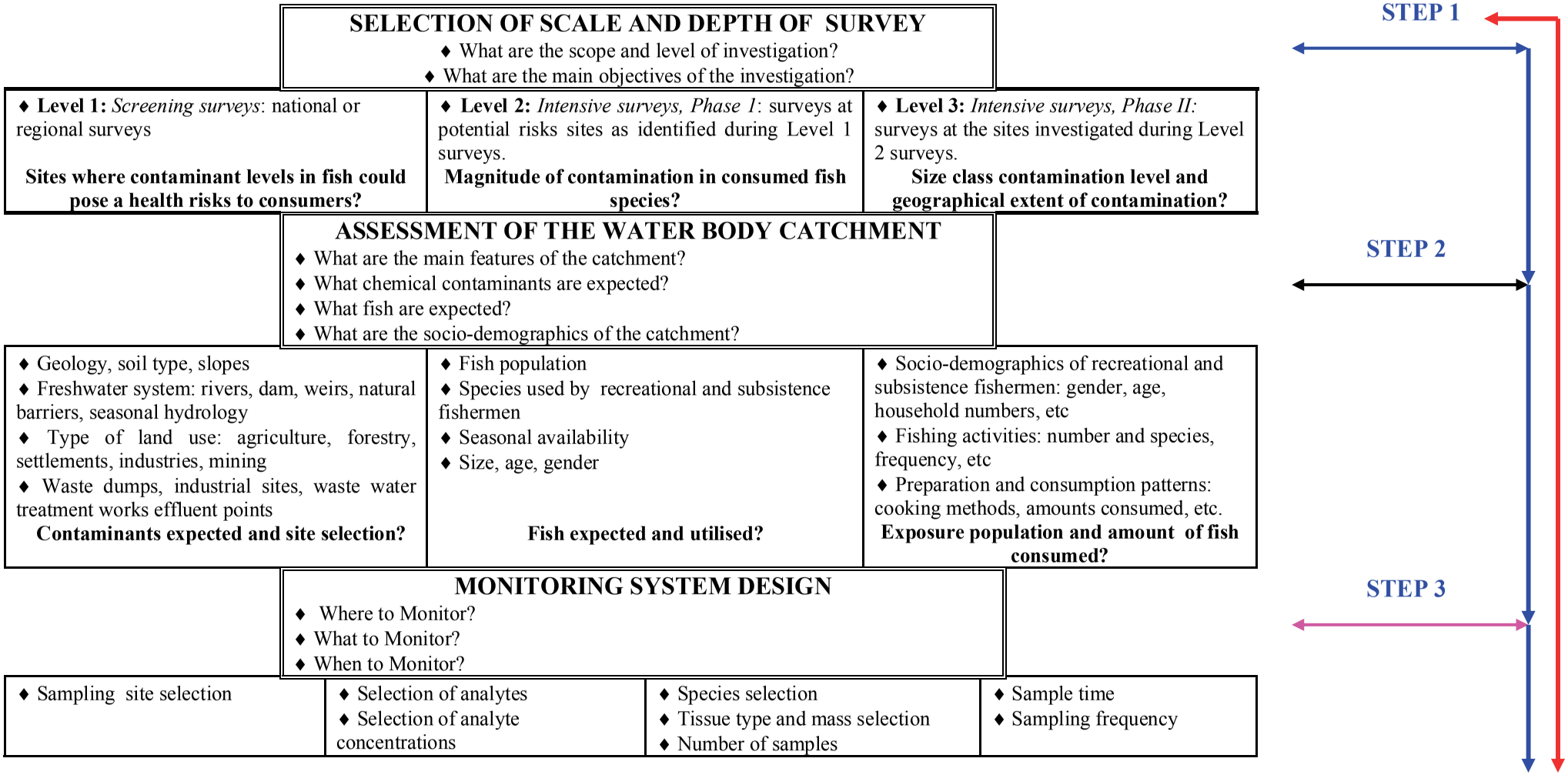


Figure 2. Protocol for freshwater fish chemical contaminant surveys for assessing the human health risks to consumers

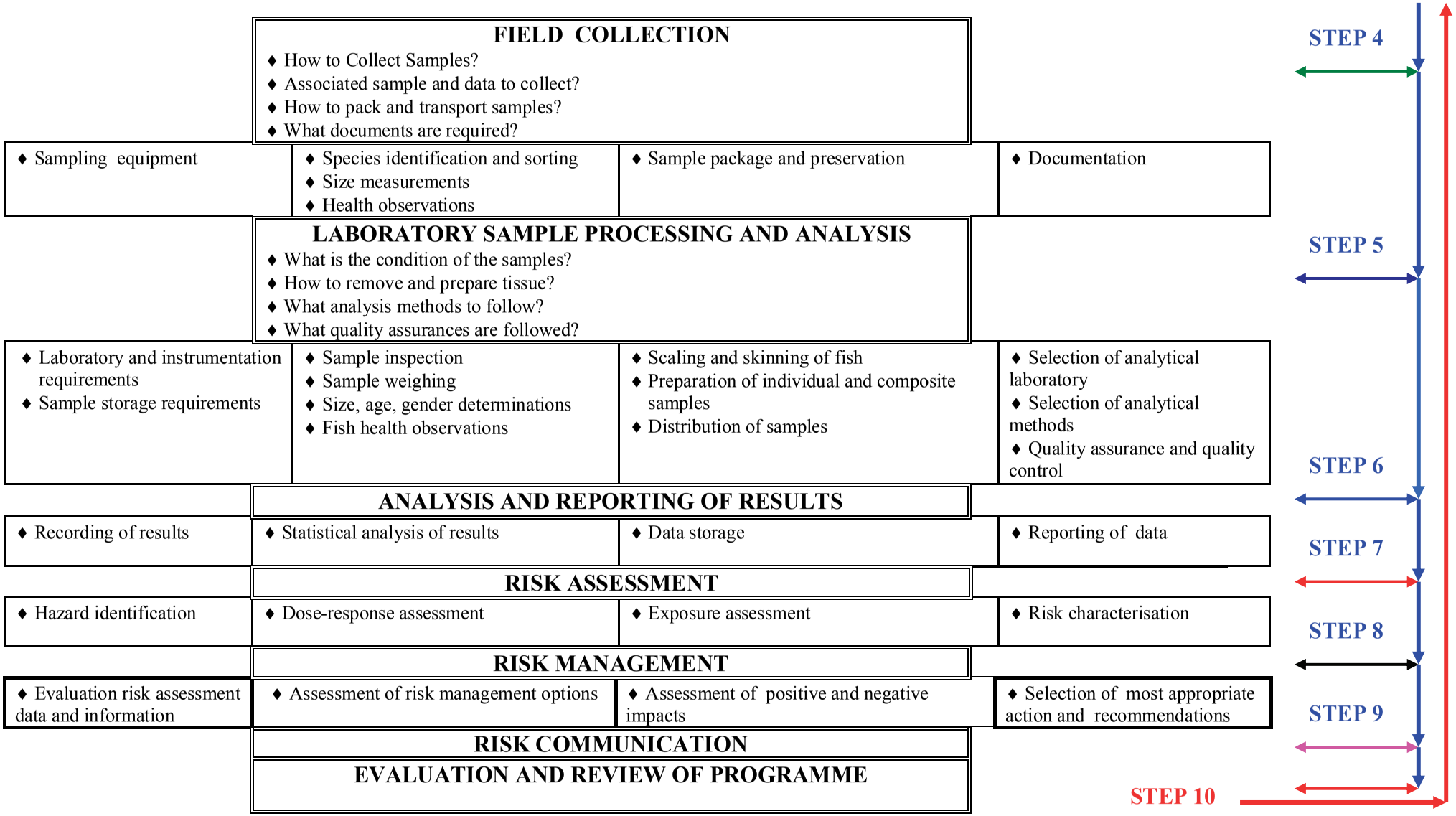


Figure 2. (Continued).

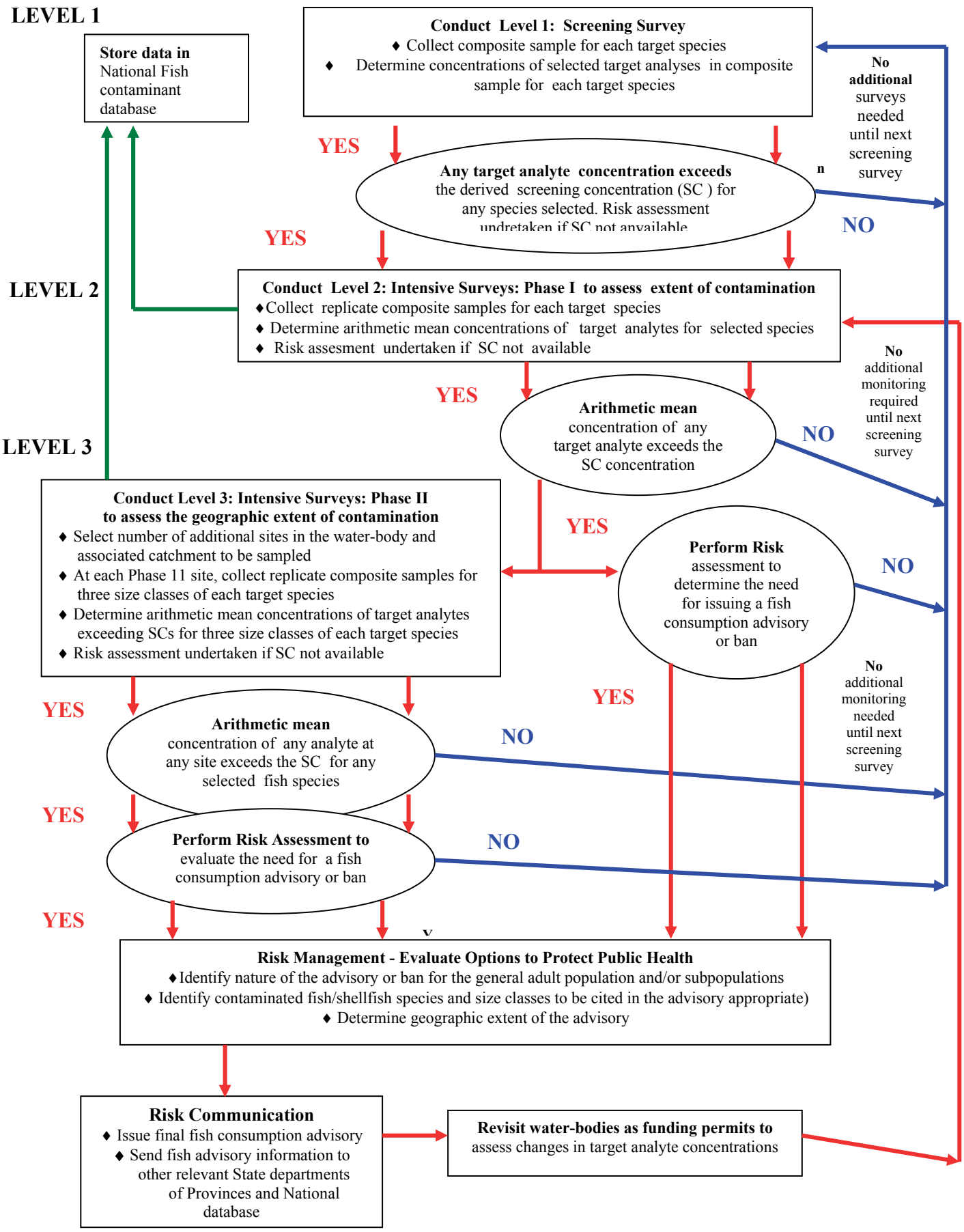


Figure 3. Monitoring levels and activities for the assessment of the human health risk associated with the consumption of chemical contaminated freshwater fish

STEP 2: ASSESSMENT OF THE WATER-BODY CATCHMENT

The water-body catchment should be assessed in order to determine the processes that drive and determine the water quality in the catchment. The general catchment characteristics (soil type topography, rainfall, hydrology, land use patterns, vegetation, etc.), anthropogenic activities and potential pollution sources are described and assessed (Figure1.2). Modelling techniques (for example GIS models, pesticide runoff models, effluent dispersal models etc.) would also aid in identifying possible problem areas and possible pollution sources (Heath, 1999).

It is essential to obtain socio-demographic information (age, sex, body mass, etc.) on the population utilising the specific water-body. Information on fish consumption patterns, for example fish species (number of species, type of fish, size classes) included in the diet, the specific edible portion selected for consumption, fish preparation and cooking methods, meal size and frequency of consuming fish by the population should be gathered. To gather this information specific fish consumption surveys can be undertaken using methods such as telephone surveys, mail surveys, personal interviews, daily record-keeping and creel census (US EPA, 1992). Not all of these techniques are valid in South Africa. Aspects such as survey design to meet the required objectives, methods for identifying participants and data collection, socio-demographic factors associated with the specific data, monitoring of quality assurance of data collection, data-processing methodology and statistical procedures for data analysis must be planned in detail before the survey is undertaken. In Table 1 a summary of the information requirements of fish consumption surveys is given, and this could be used to design these surveys. However, due to resource constraints and other practical limitations it would not be possible to obtain information on all the aspects listed. The suggested information requirements as listed in Table 1 can be adapted according to the survey objectives, the resource limitations and the local conditions. Furthermore, the general derived values (Table 2) can also be applied. Nevertheless, in countries such as South Africa, with its diversity of cultures, it is of the utmost importance that all the cultural groups are included (where appropriate) in the survey and that the methodology used to obtain information does not exclude individuals from the survey.

Table 1. Information requirements and related issues for freshwater fish consumption surveys (adapted from the US EPA, 1992).

SOCIO-DEMOGRAPHIC CHARACTERISTICS OF RECREATIONAL OR SUBSISTENCE FISHERMEN

- Age.
- Occupation/employment status.
- Income level.
- Number of household members.
- Race/ethnic group, sex, age, height and mass of the fishermen and each household member.
- Pregnancy/lactation status of women in the household.
- Language spoken at home.
- Settlement, town or city of residence.

FISHING ACTIVITIES:

- Location(s) of fishing activities (specific sites, type of water-body).
- Distance(s) of fish activities from principal residence.
- Seasonal and temporal distribution of fishing activities (total number of days per season, which months of the year, for each location).
- Fishing effort (hours/outing, hours/day, outings/month, days/month).
- Purpose of fishing (consumption, sport only: catch and return, etc.).
- Mode of fishing (nets, traps, hook and line, etc.; shore, private boat, etc.).
- Type of fish captured (general category such as bottom fish, predator, identified to species or group of species or common name).
- Numbers of fish captured per outing by species.
- Size ranges of fish captured (minimum and maximum mass and lengths by species).
- How the fish were used (released, consumed by household, sold, given away).
- Period involved in fishing activities and consuming self-caught fish (new to sport or years).

PREPARATION AND CONSUMPTION PATTERNS

- Portions of fish consumed (may vary with the species).
- How the fish were prepared for eating (skinned, fillet, steak, shucked, etc.).
- How fish were cooked (baked, fried, steamed, etc.)
- Amounts (weight) of fish caught eaten per meal/day/week/month for each person in household.
- Special cultural/ethnic practices in fish consumption and preservation.
- Consumption of fish purchased in supermarkets, fish markets, or roadside stands, etc. (amounts, frequency).
- Consumption of other aquatic organisms, waterfowl, or wildlife that may have consumed fish from same sites (amounts, frequency).
- Fish frozen or preserved and eaten throughout the year or eaten only when fresh.
- Participation in food assistance programme.
- Source of home water supply.
- Voluntary risk patterns (smoking, drinking).

Table 2. Selected input parameters for use in risk equations (adapted from the US EPA, 1997).

EQUATION PARAMETER [a]	VALUES
Maximum acceptable risk level (ARL)	10^{-4} (dimensionless) 10^{-5} (dimensionless) 10^{-6} (dimensionless)
Cancer slope factor (SF) [b] Reference dose (RFD)	$(mg/kg/d)^{-1}$ mg/kg/d
Consumer body mass (BM)	70 kg (general adult population) 70 kg (women of reproductive age) 14.5 kg (young children <6 years)
Average fish meal size (MS)	0.05 kg (children only) 0.10 kg 0.15 kg 0.25 kg 0.500 kg (adults only)
Time-averaging period (TP_{ap})	30.44 day/month (monthly limit) 14 day/14-day period (biweekly limit) 10 day/10-day period (10-day limit) 7 day/week (weekly limit)

[a] Selection of the appropriate maximum acceptable risk level, consumer body mass, and average fish meal size is considered a risk management decision.

[b] The SF [b] and RFDs values are obtained from IRIS (1999) and US EPA (1997).

[c] Values for contaminant concentrations should be determined from local fish sampling and analysis programs conducted in the water-body of concern.

STEP 3: MONITORING SURVEY DESIGN

Sampling site selection

The selection of sampling sites will vary according to the level of the survey being undertaken. It is advisable to undertake a thorough evaluation of available information (desktop survey) related to the catchment under investigation before a survey is undertaken (Heath, 1999). This will focus the study and potential sources of diffuse and point sources of pollution will be identified before sampling commences. It must, however, be stressed that potentially unpolluted sites must also be included, as they will serve as 'reference' of 'preferred state' sites.

The following should be considered:

- **Level 1:** *Screening surveys* – Depending on resources, all water-bodies where commercial, recreational or subsistence fishing are undertaken should be included. The intensity of these activities at a specific site should thus be considered. The location of the monitoring sites should be at fishing areas near point sources of pollution (e.g. industrial and municipality discharges, urban storm water drains, mine discharges etc.), diffuse sources of pollution (e.g. landfills, intensive agricultural, mining, urban development, dredging areas, etc.) and a few sites at potentially unpolluted areas. Furthermore these sited should provide information regarding the national state of the water-bodies as well water bodies that are used as drinking water

or irrigation water sources. Other considerations include (i) proximity to water and sediment sampling sites, (ii) availability of other biological data on the fish species in question, (iii) type of sampling equipment, accessibility of the site and (v) specific catchment objectives. The selection of sites can further be aided by applying techniques of surface hydrological modelling (Heath, 1999).

- **Level 2:** *Intensive surveys, Phase I* – All the sites in the Level 1 surveys where there is a potential health risk to consumers of fish. Thus the sites where the screening value for non carcinogens for one or more of the selected analytes are exceeded or potential health risk is indicated for one or more of the selected analytes using a health risk assessment tool, for example the computer software package Risk *Assistant™ (Risk *Assistant™ , 1995).
- **Level 3:** *Intensive surveys, Phase II* – The sites selected should define the geographic range of the contamination as identified during the Level 2 survey. Therefore, sites upstream and downstream of point sources of pollution and of diffuse sources of pollution are selected. Other geographical features such as barriers to migration (dams, rivers, natural waterfalls) should also be considered. The selection of specific sites will also depend on the specific impact assessment or specific case study

Selection of analytes and analyte screening concentrations

Analyte selection

To firstly protect the health of people, it is essential to select the correct analyte for inclusion in the chemical contaminant surveys. To determine the selected analytes the following procedure can be followed:

- Reviewing the data obtained from any previous contaminant monitoring surveys. In South Africa the publications by Bouwman *et al.* (1990), Grobler (1994), Claassen (1996), van Vuren *et al.* (1996), du Preez *et al.* (1997), Kotze (1997), Heath (1999). Heath & Claassen (1999) and Coetzee *et al.* 2002, to mention only a few, can be evaluated.
- Review of information on contaminants that have resulted in consumption bans or advisories. At present it is not known if any ban has been placed on the consumption of freshwater fish in South Africa. This may be due to the fact that the possible health risks associated with the consumption of freshwater fish have received little attention, although freshwater systems in South Africa are being polluted due to anthropogenic activities. Bans are usually limited to the consumption of shellfish due to the contamination by saxitoxins (Branch & Branch, 1981; WHO, 1999)**.** It is advisable to review the information of other countries on consumption advisories and bans to obtain some guidance on which chemical contaminants usually result in consumption advisories and bans. For example, information on fish and shellfish consumption advisories and bans in the United States of America can be obtained from the database "National Testing of State Fish and Shellfish Consumption Advisories and Bans" (US EPA, 1995a). An example of the contaminants from such an investigation is presented in Table 3.
- Review of analytes recommended for fish chemical contaminant monitoring. Countries may recommend specific analytes to be included in national chemical contaminant biomonitoring programmes. For example, the US EPA recommended several analytes to be investigated by the different States of the USA (Table 4).
- Review of specific standards or Acts that may stipulated the limits of contaminants in freshwater fish.

- Review of published literature and databases on the chemistry and health effect of potential contaminants (US EPA, 1995a,b). For a specific analyte the following physical, chemical and toxicological information should be evaluated:
    - Oral dose.
    - Bioaccumulation potential. For example for biocides with a bioconcentration factor greater than 300.
    - Environmental prevalence and persistence. For example for biocides with a half-life value of 30 days or more.
    - Biochemical fate of the analyte in fish.
    - Human health risks of exposure as a result of consumption of contaminated fish.
    - Analytical feasibility.
    - Permissible levels of contaminants in freshwater fish.

Electronically available databases, for example ATSDR (1998, 1999), IRIS (1999), TERA (1999), Carcinogenesis Research Information System (CCRIS) of the National Cancer Institute of the USA, Registry of Toxic Effects of Chemical Substance (RTECS) of the National Institute of Occupational Safety and Health of the USA and the Hazardous Substance Data Bank (HSDB), or risk based computer software packages, for example Risk *Assistant™ (Risk *Assistant™ , 1995) should be assessed as a vast amount of information on the human health effects of chemical contaminant are contained in these databases. The Health Effects Assessment Summary Tables (HEAST) of the US EPA (HEAST, 1992) are also important sources of information. A number of the above-mentioned databases also contain risk values for different types of chronic toxicity, for example carcinogenicity, liver toxicity and neurotoxicity. Specific publications for example Keen and Zidenberg-Cherr (1994) and US EPA (1997), also provide toxicology profiles of selected chemical contaminants. Information on the concepts of bioaccumulation and the bioaccumulation potential of chemicals can be obtained from various publications including the US EPA (1991b) and Streit (1998). Examples of some of the findings of these databases are indicted in Table 5.

From the preceding it is evident that the process of analyte selection is tedious and resource intensive. For the South African situation the following procedures are therefore recommended:

- Selected the analytes as proposed by the US EPA (1995a) as test analytes but also include the determination of lipid content of tissue.
- This list is refined if more catchment-based information of potential and actual point and/or diffuses sources of pollution becomes available, or as more analytes are identified to have negative human health effects.

Analyte concentration

A screening concentration (SC: the concentration of a selected analyte in fish tissue that is of potential concern to consumers from a health perspective and which is used as a standard against which levels of contamination, in similar tissue collected from the freshwater environment to which it can be compared to) must be derived (US EPA, 1995a). The US EPA (1995a) recommends that a risk-based approach is followed for deriving screening concentrations for the following reasons:

- The priority is protection of public health.
- It provides a direct link between fish consumption rate and risk levels.
- The estimate of increased risks is usually conservative.
- It is designed for the protection of consumers of locally captured fish, for example, recreational or subsistence fishermen who are at potentially greater risk than the general population.
- It is the basis for developing water quality criteria.

Two different risk base models are used to derive screening concentrations for analytes as fundamental differences exist between the carcinogenic and non-carcinogenic dose-response variables. The SC is therefore calculated for both carcinogenic and non-carcinogenic (US EPA, 1995a).

Table 3. Chemical contaminates that resulted in fish and shellfish advisories in the United States of America (adapted from US EPA, 1995a).

CONTAMINANT	CONTAMINANT
Metals Arsenic (total) Cadmium Chromium Copper Lead Mercury Selenium Zinc Organometallics	**Pesticides** Chlordane DDT and metabolites Dieldrin Heptachlor epoxide Hexachlorobenzene Kepone Mirex Photomirex Toxaphene
Polycyclic aromatic hydro-carbons (PAHs)	**Polychlorinated biphenyls (PCBs)**
Other chlorinated organics Dichlorobenzene Hexachlorobutadiene Pentachlorobenzene Pentachlorophenol Tetrachlorobenzene Tetrachloroethane	**Others** Creosote Gasoline Multiple pollutants Phthalate esters Polybrominated biphenyls (PBBs)
Dioxins/furans	

Table 4. Analytes that have been recommended by the US EPA for fish and shellfish chemical contaminate monitoring in the United States of America (US EPA, 1995a). These analytes are also recommended for freshwater fish chemical contaminate monitoring in South Africa.

Metals and metalloids
Arsenic
Cadmium
Chromium
Lead
Manganese
Mercury
Selenium
Copper
Zinc
Organochlorine Pesticides
Total DDT (sum of 4,4- and 2,4- isomers of DDT, DDE, and DDD)
Carbofuran
Dieldrin
Endosulfan (I and II)
Lindane (ϒ-hexachloro-cyclohexane; ϒ-HCH)
PCBs
Total PCBs (sum of Aroclors)

Table 5. Example of potential hazards of the variables selected for recommended for freshwater fish chemical contaminate monitoring in South Africa.

POTENTIAL HAZARD DESCRIPTIONS OF VARIABLES
DDT • High levels of DDT can affect the nervous system causing excitability, tremors and seizures. The effects are reversible once exposure stops. • Studies in animals have shown exposure to DDT can cause liver cancer (however, this was not shown in studies of DDT-exposed water). • EPA have classified DDT as a 'probable human carcinogen'.
PCBs (Polychlorinated biphenyls) • (The major dietary sources of PCBs are fish, meats, and diary products) • High levels of PCBs may cause skin conditions such as acne and rashes. Effects caused by PCBs in animals include reductions in the immune system function, behavioral alterations and impaired production. • Studies of women who consumed high amounts of fish contaminated with PCBs had babies that weighed less than babies from women who did not eat fish. In addition, babies born to women exposed to high amounts of fish contaminated with PCBs before and during pregnancy shared abnormal responses to tests of infant behavior.
MERCURY • At high levels may damage the brains, kidneys and developing foetus. The US EPA has classified mercuric chloride and methyl mercury as 'possible human carcinogen'.
CARBOFURAN • Causes reproductive effects. It is not known whether it is carcinogenic.
DIELDRIN • Causes liver damage? And it is classified as a 'probable human carcinogen'.
ARSENIC • Large oral doses of arsenic can result in death and over doses (0,3 – 30 ppm) may cause irritation of stomach and intestines, with symptoms including stomach ache, nausea, vomiting and diarrhea. Other effects may include decreased production of red and white blood cells which may result in fatigue, abnormal heart rhythm, blood-vessel damage resulting in bruising and impaired nerve function causing 'pins and needle' sensation in the hands and feet. • Skin changes may also occur as well as skin cancer, increased risk of cancer in the liver, bladder, kidneys prostrate and lungs.
CADMIUM • Can cause kidney disease and may irritate the digestive tract. Other long-term effects are lung damage and fragile bones. Animal studies have shown that more cadmium is absorbed into the body if the diet is low in calcium, protein or iron, or is high in fat. Cadmium may reasonably be 'anticipated to be a carcinogen'.
LEAD • Can damage the nervous system, kidneys and reproductive system. It can affect almost every organ and system in the body, but the most sensitive is the central nervous system, particularly in children. At high levels lead may decrease reaction time, weakness in fingers, wrists or ankles and possibly affect memory. It may cause anaemia, as well as damage the male reproductive system. Based on animal studies, lead may reasonably be 'anticipated to be carcinogenic'.

The following equation is used to calculate screening concentrations for carcinogenic contaminants:

$$\mathbf{SC_c = [(RL/SF) \times BM]/CR} \qquad \mathbf{(1)}$$

where:

- SCc = Screening concentration for a carcinogen (mg/kg).
- RL = Maximum acceptable risk level (dimensionless). This is the assigned level of maximum acceptable risks over an individual's lifetime for example RL = 10^{-4} for a level of risk not to exceed one excess case of cancer per 10 000 individual exposed over a 70-year lifetime.
- SF = Oral slope factor or carcinogenicity potency factor $(mg/kg/day)^{-1,}$ which is an upper bound risk value. The slope of the dose-response curve in the low-dose region used with exposure to calculate the estimated lifetime cancer risk. Most often expressed as risk per milligram of exposure to the toxic chemical per body mass per day. This is usually calculated using the upper 95% confidence limit on the linear term in the linearised multistage model.
- BM = Mean body mass of the general population or subpopulation of concern (kg).
- CR = Mean daily consumption rate of the species of interest by the general population or subpopulation of concern averaged over a 70-year lifetime (kg/day).

For the calculation of screening concentrations (SCn) for non-carcinogenic contaminants the following equation is used:

$$\mathbf{SCn = (RFD \times BM) / CR} \qquad \mathbf{(2)}$$

where:

- SCn = Screening concentration for a non-carcinogenic (mg/kg).
- RFD = Oral reference dose (mg toxicant/kg human body mass/day). This is an estimate of the daily exposure of the human population that is likely to be without appreciable risks of deleterious effects during a lifetime. The RFD is derived by applying uncertainty or modifying factors to a sub-threshold dose determined during chronic animal bioassay. For example to the LOAEL (lowest exposure level at which there are statistical or biologically significant increases in frequency of severity of adverse effects between the exposure population and its appropriate control group) is used if the NOAEL (exposure level at which there are statistical or biologically significant increases in the frequency of severity of adverse effects between the exposure population and its appropriate control group) is not determined. The uncertainty or modifying factors are used to account for the following uncertainties in:
    - Sensitivity differences between different human populations.
    - Extrapolation from animal data to humans.
    - No human data is available.
    - Deriving RFD from LOAEL when NOAEL is not available.
    - Incomplete or inadequate toxicological or pharmacokinetic information.

These factors range from 1 to 10 for each factor (Table 6) and the final uncertainty and modifying factor (UF **x** MF) which is determined by multiplying the uncertainty (UF)

modifying UF x MF value. The RFD is then derived by deriving the NOAEL or the final calculated uncertainty and modify value:

(RFD = NOAEL or LOAEL / (UF **x** MF).

To obtain calculated oral RFD and/or SF's for chemical contaminants the Integrated Risk Information System (IRIS, 1999) electronic data base, can be assessed. This databases contains health-risk and EPA information on more that 400 different chemicals (US EPA, 1995a,b).

The values for body mass and consumption rates should be determined for the general adult population and the specific sub-population in question. The US EPA use a BM = 70 kg and a mean daily consumption rate of 6.5 g/d to calculate the SF's for a general adult population. The CR value may be too low for a specific recreational or subsistence fishing population as it represents a consumption rate for the average consumer of fish in a general adult population (US EPA, 1995b). To address uncertainty (Table 5) the risk level factors for the calculation of screening concentrations is 10^{-5}; however, values may range from 10^{-4} to 10^{-5}. Thus for the deriving of screening concentrations the following values are recommended:

- Body mass (BM) 70 kg, average adult's body mass.
- Mean daily consumption rate (CR for non-carcinogens = 6.5 g/day).
- Maximum acceptable risk level (RL for carcinogens = 10^{-5}).

The stated procedure was used by the US EPA (1995) to derive screening concentration and the dose-response variable used to derive them for the selected analytes (Table 5). It must, however, be emphasised that depending on the availability of data other similar equations can also be used to calculate screening concentrations (US EPA, 1991).

The following equation can be used for calculating screening concentrations for carcinogenic contaminants:

$$\mathbf{SC_c = (RL \times BM)/ SF \times \{[WI/(BCF \times FM \times LR)] + CR\}} \quad \mathbf{(3)}$$

where:

- SCc = Screening concentration for a carcinogen (mg/kg).
- RL = Maximum acceptable risk level (dimensionless). This is the assigned level of maximum acceptable risks over an individual's lifetime: for example RL = 10^{-4} for a level of risk not to exceed one excess case of cancer per 10 000 individuals exposed over a 70-year lifetime.
- BM = Mean body mass of the general population or subpopulation of concern (kg).
- SF = Oral slope factor or carcinogenicity potency factor $(mg/kg/day)^{-1}$, which is an upper bound risk value.
- WI = Mean adult water intake (2 litres/day).
- BCF = Bioconcentration factor (mg toxicant/kg fish divided by mg toxicant/litre water) for fish with 3 percent lipid.
- FM = Food chain multiplier.
- LR = Ratio of lipid fraction in fish tissue assumed to be 3 percent.

- CR = Mean daily consumption rate of the species of interest by the general population or subpopulation of concern averaged over a 70-year lifetime (kg/day).

Table 6. Uncertainty factors and modifying factors for estimating exposure limits (adapted from the US EPA, 1997).

UNCERTAINTY OR MODIFYING FACTOR	GENERAL COMMENTS	STANDARD VALUE
Uncertainty factor: human (intraspecies)	Used to account for the variability of response in human populations.	10
Uncertainty factor: animal to human (interspecies)	Used to account for differences in responses between animal study species and humans.	10
Uncertainty factor: data gaps	Used to account for the inability of any study to consider all toxic endpoints. The intermediate factor of 3 (1/2 log unit) is often used when there is a single data gap exclusive of chronic data.	3 to 10
Uncertainty factor: LOAEL to NOAEL	Employed when a LOAEL instead of a NOAEL is used as the basis for calculating an exposure limit. For "minimal" LOAELs, an intermediate factor of 3 may be used.	3 to 10
Modifying factor	Has been used for differences in absorption rates, tolerance to a chemical, or lack of sensitive endpoint. The default value is 1.	1 to 10

LOAEL = Lowest observed adverse effects level.
NOAEL = No observed adverse effects level.

For the calculation of screening concentrations (SCn) for non-carcinogens the following equation can also be used:

$$\mathbf{SCn = [(RFD \times BM) - (DT + IN) \times BM]/ [WI/ (BCF \times FM \times LR)] + CR} \qquad \mathbf{(4)}$$

where:

- SCn = Screening concentration for a non-carcinogen (mg/kg).
- DT = Daily exposure, excluding fish (mg toxicant/kg human body mass/day).
- IN = Inhalation exposure (mg toxicant/kg human body mass/day).
- RFD = Oral reference dose (mg toxicant/kg human body mass/day) as defined as in Equation 2.
- BM = Mean body mass of the general population or sub-population of concern (kg).
- WI = Mean adult water intake (2 litres/day).
- BCF = Bioconcentration factor (mg toxicant/kg fish divided by mg toxicant/litre water) for fish with 3 percent lipid.
- FM = Food chain multiplier.
- LR = Ratio of lipid fraction in fish tissue assumed to be 3 percent.
- CR = Mean daily consumption rate of the species of interest by the general population or subpopulation of concern averaged over a 70-year lifetime (kg/day).

To calculate screening concentrations for South Africa scenarios it is recommended that the above-mentioned procedure of the US EPA (1995a) is used. The screening concentrations as listed in Table 5 should be used if data (for example on body mass and/or concentrations rates etc) are not available to modify them. The following is therefore recommended for the South African surveys:

- **Level 1:** *Screening surveys* – Monitor for the selected analytes as listed in Table 5 Refine the list as more catchment-based analyte concentrations and/or information or additional toxicological data for other analytes becomes available. Use the screening values as listed in Table 5 and adapt these values as more information regarding the local population becomes available. Alternatively, use the obtained chemical contaminant concentrations directly in the Risk *Assistant^{TM} software package.
- **Level 2:** *Intensive surveys, Phase I* – Monitor the selected analytes that exceed the screening concentration. Chemical contaminant concentrations just below or at the screening concentration should be re-assumed to determine if they must be further monitored. The same screening concentrations as in Level 1 surveys are used and are only modified if more local population information becomes available. Alternatively use the obtained chemical contaminant concentrations directly in the Risk *Assistant^{TM} software package.
- **Level 3:** *Intensive surveys, Phase II*: – The same recommendations as for Level 2 surveys, but a broader geographical area must be surveyed and different size classes of a specific specie are selected for evaluation. The recommendations for Level 2 survey would also hold for impact assessments or during specific case studies The obtained chemical contaminant concentrations are directly applied in the Risk *Assistant^{TM} software package.

Species selection

In South Africa various freshwater species have been used to investigate the levels of selected biocides and metals (Table 7). Ideally, species from two distinct ecological groups of fish (e.g. bottom feeders and predators) which are known to bioaccumulate high concentrations of chemical contaminants over a wide geographic range should be used (US EPA, 1995a; Heath, 1999). Bottom-feeding species are important as they are in direct physical contact with sediment and/or consume benthic invertebrates and epibenthic organisms that are in the sediment. Due to the varying geographical distribution and environmental requirements of each species it is impossible to sample the same species at every selected site in South Africa. However, a limited
number of species should be identified that are distributed widely enough to allow for collection and comparison of contaminants data from many sites (US EPA, 1995a).

When selecting fish species the following criteria should be considered:

- The species are commonly consumed in the study area and are thus of commercial, recreational or subsistence fishing importance.
- The species have the potential to bioaccumulate high concentrations of pollutants.
- The distribution of the species is relative wide and is easily identified taxonomically.
- The species are relatively abundant and easy to capture.
- The species are large enough to provide adequate tissue samples for analysis (US EPA, 1995a).

However, due to the patchy distribution of South African species and limited commercial fishing activities, little knowledge about the bioaccumulation potential of species has been gathered. In addition, the absence of published data on the quantitative utilisation of South African species makes it difficult to select specific species for recommended use. Nevertheless, based on the information in Table 7 the fish species listed in Table 8 are recommended as selected fish species and should give some guidance as to the selection of fish species for a specific region. Based on the findings of the US EPA (1995a), Heath (1999) and the data summarised in Table 8 the following is recommended:

- **Level 1:** *Screening surveys* – At least one bottom feeder or one predator species selected from the species in Table 8. Preferably include one bottom feeder and one predator species.
- **Level 2:** *Intensive surveys, Phase 1* – Include the same species as for the Level 1 surveys but include more species if they are captured in sufficient numbers and funds are available.
- **Level 3:** *Intensive surveys, Phase II.* – The same recommendation as for Level 2 surveys.

It must be emphasised that the final selection must also include information provided by the local Governmental Official responsible for freshwater fish and local human consumption data.

Table 7. South African freshwater fish species that may be considered for chemical contaminant investigations. Scientific and common names as well as importance to recreational and subsistence fishermen are based on information from Skelton (2001).

FAMILY NAME	SCIENTIFIC NAME	COMMON NAME	CAPTURED BY ANGLERS	CAPTURED BY SUBSISTANCE FISHERMEN	USED IN CON-TAMINANT STUDIES
MORMYRIDAE	*Marcusenius macrolepidotus*	Bulldog	4	4	
	Petrocephalus catostoma	Churchill		4	
CYPRINIDAE	*Barbus mattozi*	Papermouth	4	4	
	Barbus serra	Sawfin	4	4	
	Labeobarbus andrewi	Whitefish	4	4	
	Barbus kimberleyensis	Largemouth yellow fish	4	4	4
	Labeobarbus aeneus	Smallmouth yellow fish	4	4	4
	Barbus aotalensis	Scaly	4	4	
	Labeobarbus polylepis	Smallscale yellow fish	4	4	4
	Barbus capensis	Clanwilliam yellow fish	4	4	
	Barbus marequensis	Large scale yellow fish	4	4	4
	Labeo umbratus	Moggel	4	4	4
	Labeo capensis	Orange River mudfish	4	4	4
	Labeo rubromaculatus	Tugela labeo	4	4	
	Labeo rosae	Rednose labeo	4	4	4
	Labeo rudi	Silver labeo	4	4	
	Labeo congoro	Purple labeo	4	4	4
	Labeo seeberi	Clanwilliam sandfish	4	4	
	Labeo molybdinus	Leaden labeo	4	4	4
	Cyprinus carpio	Carp	4	4	4
CHARACIDAE	*Brycinus imberi*	Imberi			4
	Brycinus lateralis	Striped robber		4	
	Hydrocynus vittatus	Tigerfish	4	4	4
SCHILBEIDAE	*Schilbe intermedius*	Silver catfish or Butter barbel	4	4	4
CLARIIDAE	*Clarias gariepenus*	Sharptooth catfish	4	4	4
MOCHOKIDAE	*Synodontis zambezensis*	Brown squeaker	4	4	4
SALMONIDAE	*Salmo trutta*	Brown trout	4	4	
	Oncorhypnchus mykiss	Rainbow trout	4	4	

Table 7. Continued.

FAMILY NAME	SCIENTIFIC NAME	COMMON NAME	CAPTURED BY ANGLERS	CAPTURED BY SUBSISTANCE FISHERMEN	USED IN CON-TAMINANT STUDIES
CENTRACHI-DAE	*Micropterus salmoides*	Largemouth bass	4	4	
	Micropterus dolomieu	Smallmouth bass	4	4	4
	Micropterus punctulatus	Spotted bass	4	4	
PERCIDAE	*Perca fluviatilis*	Perch	4	4	
CICHLIDAE	*Chetia flaviventris*	Canary kurper	4	4	
	Tilapia sparrmanii	Banded	4	4	
	Peudocrenila-brus philander	Tilapia southern mouth brooder			4
	Tilapia rendalli	Redbreast tilapia	4	4	4
	Oreochromis mossambicus	Mozambique	4	4	4
	Oreochromis placidus	Black tilapia	4	4	

Species size class selection

Some correlation between increasing size (age) of the fish and contaminant concentration has been recorded (Streit, 1998). If the aim is to link a fish advisory to a specific fish size class while the other size classes of the selected species remains open, then fish in specific size classes must be analysed. For example, if the contaminant concentrations are positively correlated with fish size (age) consumption of the smaller individuals may be acceptable even though the larger size classes may be restricted.

The following is recommended:

- **Level 1:** *National screening surveys* – If resources are limited, collect only one size class for each of the selected species and focus on the larger size class commonly consumed. Preferably collect individuals from three size classes, which include the exposure and consumption size ranges.
- **Level 2:** *Intensive surveys, Phase 1* – Collect individuals from three size classes covering the exposure and consumption ranges. Select more size classes if more refinement in the relationship between size classes and advisories is required.
- **Level 3:** *Intensive surveys, Phase II* – The same as for Level 2: Phase I surveys.

Table 8. Freshwater fish species that are recommended for consideration for chemical contaminant investigations in South Africa.

FAMILY NAME	SCIENTIFIC NAME	COMMON NAME	FEEDING HABITS
CYPRINIDAE	*Labeobarbus aeneus*	Small mouth yellow fish	Bottom feeder, omnivorous
	Barbus andrewi [1]	White fish	Bottom feeder, invertebrates and algae
	Labeobarbus natalensis [2]	Scaly	Omnivorous, algae, invertebrates, detritus
	Labeobarbus polylepis	Small scale yellowfish	Carnivorous; algae; and invertebrates
	Labeo capensis	Orange River mudfish	Bottom feeder, omnivorous; algae and invertebrate
	Labeo molybdinus	Leaden labeo	Algae eater from rocks
	Labeo rosae	Rednose labeo	Detritivore; bottom feeder, invertebrates in sediments
	Labeo rubromaculatus [2]	Tugela labeo	Detritivore; bottom feeder, algae and detritus
	Labeo umbratus [3]	Moggel	Detritivore; bottom feeder, soft mud and detritus
	Cyprinius carpio[4]	Carp	Omnivore; bottom feeder
SCHILBEIDAE	*Schilbe intermedius*	Silver catfish/Butter barbel	Omnivorous; middle and surface water feeder
CLARIIDAE	*Clarias gariepinus*	Sharptooth catfish	Omnivorous
SALMONIDAE	*Oncorhynchus mykiss*[3,4]	Rainbow trout	Carnivorous predator; feed on invertebrates, fish, frogs
CENTRARCHIDAE	*Micropterus salmoides*[4]	Largemouth bass	Carnivorous; predator invertebrates, frogs, fish
	Micropterus dolomieu [4]	Smallmouth bass	Carnivorous; predator, feeds on invertebrates, fish
CICHLIDAE	*Oreochromis mossambicus*	Mozambique tilapia	Omnivorous, algae detritus invertebrates
	Tilapia sparrmanii [5]	Banded tilapia	Omnivorous, feeds on algae and invertebrates
	Tilapia rendalli	Redbreast tilapia	Algae and plant eater but also include invertebrates

1. Distribution confined to: Western Cape Province
2. Distribution confined to: Kwazulu Natal Province
3. Important commercial specie
4. Exotic specie
5. Mainly important to subsistence fishermen

Tissue type and mass selection

The studies reviewed for South African fish revealed that different tissue from individual species is usually analysed. To make effective use of the data containing the chemical contaminant levels in fish for the protection of human health the tissue samples should consist of the portion of the fish that is consumed by the population under investigation (US EPA, 1995a). For South African conditions it is assumed that people usually gut the fish and that fillets are usually consumed. Fillets with skin on (including the belly flap) but with the scales removed are recommended for most scaled freshwater fish. However, the analysing of skinless fillets must be considered if the complete homogenisation of skin-on fillets is not achievable or if the local consumers only prepare skinless fillets.

The methods of sample removal are discussed in more detail under 'Scaling and skinning fish'. For scaleless fish species, for example the African sharptooth catfish (C*larias gariepinus)*, the skin should be removed (US EPA, 1995a). However, in some communities whole fish (especially if they are small) are consumed. The selection of sample type should thus be adapted to individual local consumption preferences. Although internal organs are usually not consumed the analysis of liver, fat and gonad tissues should be included as some analytes is lipophilic and tends to bioaccumulate in these tissues. However, a precise description of the tissue type used is essential.

The use of composite tissue samples made of tissues from individuals from the same species are recommended because:

- Composite samples are more cost-effective for estimating mean tissue concentrations.
- Adequate sample mass is available for selected analyte analysis (Table 9) at appropriate detection limits.
- Adequate sample mass for quality assurance and quality control requirements for the analysis of replicate, matrix and duplicate specie samples is obtained.
- Re-analyses of tissue samples are possible (US EPA, 1995a).

The following information will, however, be gained if the fillets of individual fish are analysed:

- Thus provide a direct measure of the range and variability of chemical contaminant concentrations in the selected fish populations.
- Data on the possible maximum contaminant concentrations are provided which can be used to evaluate acute human health risks.
- Variability of contaminant concentrations among individual fish provides data that can be used to derive the desired statistical objectives of the survey.

If the tissue from individual fish for a specific composite sample is kept separate it can be analysed individually, for example if a contaminant concentration in the composite of the tissue is higher or close to the pre-determined health risk values. Fish used in a composite sample must fulfil the following requirements:

- Must be of the same species.
- Must be of similar size, therefore the total length of the smaller individuals must not be less than 75 percent of the total length of the largest individual. Pre-determined size classes should be taken into account.

Table 9. Sample mass of individual homogenates required for Level 1 surveys (adapted from US EPA, 1995a)

NUMBER OF FISH PER SAMPLE	TOTAL COMPOSITE MASS (wet mass)		
	100 g	200 g	500 g
3	33	67	167
4	25	50	125
5	20	40	100
6	17	33	84
7	14	29	72
8	13	25	63
9	11	22	56
10	10	20	50

- The mean total length (size) of fish within a composite sample for a specific site and the mean of the mean lengths of fish in all of the composite samples collected from the specific site should not exceed 10%. For example, if the mean total lengths of fish in five composite samples are 380, 420, 400, 420, and 430 mm respectively, the mean length (± 10 %) of fish in the five replicates is 410 ± 41 mm. The mean length of individual fish in each of the five replicate samples should be within 369 to 451 mm range.
- Must be collected at the same time to ensure that temporal changes in concentrations, for example associated with the reproductive cycle of the fish is minimised. The individual fish used in a composite sample must therefore be collected within 7 days.
- Must be in the size classes that are consumed.
- Must be in sufficient numbers to provide the required tissue mass. Heath (1999) recommended that at least 20 g wet mass for metal analysis is removed from individual fish (five individuals should be selected). However, this would be only provide 100 g of sample which would be too little if all the analytes must be analysed for.

Based on the US EPA guideline the following is recommended:

- **Level 1:** *Screening surveys* – A 200 g wet mass composite sample of edible-scaled skin-on or skinless (for fish without scales) fillets, liver fat and gonad tissues should be collected. Analyzing of skinless fillets must be considered if the complete homogenisation of skin-on fillets is not achievable or if the local consumers only prepare skinless fillets. Each composite sample should consist of eight individual fish; therefore each individual should contribute 25 g wet mass to the composite (Table 9). However, a large composite mass may be required if the number of analytes is increased to address specific concerns or if the analytical procedures of the specific laboratory require a larger tissue mass. The same number of individual fish must be used in each composite sample for a selected species.

- **Level 2:** *Intensive surveys, Phase 1* – The same as for Level 1 surveys, but the mass can be reduced if the number of selected analytes of concern are reduced as a result of data obtained during Level 1 surveys.
- **Level 3:** *Intensive surveys, Phase 11* – The same recommendations as for Level 2 surveys.

Number of samples to be taken

The overall objective in selecting the appropriate number of replicate composite samples per selected site (n) and the number of individual fish per composite sample (m) is to test the nul hypothesis, H_o and the alternative hypothesis, H_A, where:

> H_o = the mean selected analyte concentration of replicate composite samples at a site is equal to the screening concentrations (SC).
> H_A = the mean selected analyte concentration of replicate composite samples at a site is greater than SC.

According to the US EPA (1995a) the best sampling design would specify the minimum number of replicate composite samples (n) and the number of fish per composite sample (m) that would detect a minimum difference between SC and the mean selected analyte concentration of replicate samples at a site. Such a sampling design should be based on:

- The minimum detectable difference between the site-specific mean selected analyte concentration on the SC.
- The level of significance that is the probability of rejecting the H_o when a difference does not exists.
- Population variance (σ^2) that is the variance in the target analyte concentrations among individuals from the same species of fish. The following statistical model can thus be derived:

$$\mathbf{Var}\ (\bar{z}) = \sigma^2/nm$$

Where:
- $\bar{z}$ = the mean selected analyte concentration of selected replicate composite samples at the site
- σ^2 = population variance
- n = number of replicate composite samples
- m = number of individual samples in each composite sample

For the H_o and the H_A the estimate of Var ($\bar{z}$), s^2 for a composite sample is:

$$s^2 = [\sum (Z_i - \bar{z})^2]/[n(n-1)]$$

where:
- s^2 = the estimate of Var ($\bar{z}$) for a composite sample.
- $\bar{z}$ = the mean selected analyte concentration of selected replicate composite samples at the site
- Z_I = contaminant concentration of the replicate composite sample at the selected site where i = 1,2,3,4…n.
- n = number of replicate composite samples

Under the H_o:

$$(\bar{z} - SC)/s$$

where:

- the statistic has a Student t distribution with (n-1) degrees of freedom.
- the degrees of freedom are one less than the number of composite samples
- $\bar{z}$ = the mean selected analyte concentration of selected replicate composite samples at the site
- SC = screening concentration
- s = variance

- Power of the hypothesis test; that is, the probability of detecting a true difference when one exists.
- Cost involved: for example, sample collection, sample preparation, analysis cost and general overheads.

A minimum of three replicate composite samples should be collected at each site. This approach reduces the risk of not having at least two replicate composite samples for the estimation of variance at a specific site.

If the above design specifications are not available an investigation of the statistical precision (measure of the stability of estimate) of the estimate σ^2/nm and of statistical power should be undertaken. This would give some indication for the selection of the number of replicate composite samples at the selection site and the number of fish per composite sample (US EPA, 1995a).

The selected analyte concentration tends to be normally distributed if composite samples are analysed. The standard error (se) of σ^2/nm is thus derived as:

$$\mathbf{se\ [\sigma^2/nm] = \sigma^2\ \ [\ 2/n^2m^2(n-1)]^{\frac{1}{2}}}$$

In the equation above the function of n and m is shown in square brackets. The standard error can be used to determine what the statistical improvement would be if the number of replicates composite samples and/or the number of fish within a composite sample is changed. For example, if the number of fish per composite sample is fixed, the standard error of σ^2/nm will decrease as the number of replicate samples increase. Furthermore, a greater precision in the estimated standard error of $\Downarrow$ is obtained by increasing the number of replicate samples than by increasing the number of fish per composite sample. For example, if 60 fish have to be captured, a design of 10 replicate samples of 6 fish each, the value of the function of n and m will be 0.008 and for a design of 6 replicate composite of 10 fish each the value would be 0.012 (Table 10). Therefore, there is a greater precision in the estimated standard error of $\Downarrow$ associate with the former design as compared to the latter design.

To derive the statistical power for the H_o in question (Table 11) the following assumptions was made:

- the ratio of the stimulated population deviation to the screening concentration (σ/SC) is 50 or 100 percent.

Table 10. Values [$2/n^2m^2(n-1)]^{½}$ of different combination of n and m (adapted from US EPA, 1995a).

No. of replicate composite samples (n)	Number of fish per composite sample (m)									
	3	4	5	6	7	8	9	10	12	15
3	0.111	0.083	0.067	0.056	0.048	0.042	0.037	0.033	0.028	0.022
4	0.068	0.051	0.041	0.034	0.029	0.026	0.023	0.020	0.017	0.014
5	0.047	0.035	0.028	0.024	0.020	0.018	0.016	0.014	0.012	0.009
6	0.035	0.026	0.021	0.018	0.015	0.013	0.012	0.011	0.009	0.007
7	0.027	0.021	0.016	0.014	0.012	0.010	0.009	0.008	0.007	0.005
10	0.016	0.012	0.009	0.008	0.007	0.006	0.005	0.005	0.004	0.003
15	0.008	0.006	0.005	0.004	0.004	0.003	0.003	0.003	0.002	0.002

Table 11. Estimates of statistical power of hypothesis of interest under specified assumptions (adapted from US EPA, 1995a).

No. of replicate composite samples (n)	Number of fish per composite (m)									
	3	4	5	6	7	8	9	10	12	15
A. Ratio of ó/SC = 0.5 and µ = 1.5 x SC:										
3	6	6	7	8	9	9	9	9	9	9
4	8	9	9	9	9	9	9	9	9	9
5	9	9	9	9	9	9	9	9	9	9
6	9	9	9	9	9	9	9	9	9	9
7	9	9	9	9	9	9	9	9	9	9
10	9	9	9	9	9	9	9	9	9	9
15	9	9	9	9	9	9	9	9	9	9
B. Ratio of ó/SC = 1.0 and µ = 1.5 x SC:										
3	-	-	-	-	-	-	-	-	5	6
4	-	-	-	5	6	6	7	7	8	8
5	-	5	6	7	8	8	8	8	9	9
6	5	6	7	8	8	8	9	9	9	9
7	6	7	8	8	9	9	9	9	9	9
10	8	8	9	9	9	9	9	9	9	9
15	9	9	9	9	9	9	9	9	9	9

- : Power less than 50 percent
5: Power between 50 and 60 percent
6: Power between 60 and 70 percent
7: Power between 70 and 80 percent
8: Power between 80 and 90 percent
9: Power above 90 percent

- the true mean of the site specific composite selected analyte concentrations (μ) is 50 percent higher than the screening concentrations.

The following can be conducted from the estimated statistical power (Table 11) of the H_o in question:

- The statistical power increases if the number of replicate composite samples (n) and/or the number of fish per composite sample increase.
- A greater increase in statistical power is achieved by increasing the number of replicate composite samples than increasing the number of fish per composite.
- The statistical power decreases if the number of replicate composite samples per site and the number of fish per composite are kept constant but the ratio of the estimated population variance to the SC increases (σ/SC).
- The assumption regarding the ratio of σ/SC in Section A of Table 11 may be unrealistic for some fish populations.
- The estimates in Section B of Table 11 are based on more realistic assumption but shows that only large differences between the site–specific mean selected analyte concentrations and the SC will be detected.

When multiple analytes are selected the selected analyte with the largest population variation should be used to determine the number of replicate composite samples per site and the number of fish per sample (US EPA, 1995a).

In South Africa factors such as the low abundance and availability of fish in some rivers and financial constrains may limit the number of samples collected (Heath, 1999). Heath (1999) therefore suggested that five individual fish samples should be collect for human health assessment**.**

Based on some of the guidelines of the US EPA (1995), the findings of Heath (1999), Du Preez 2000 and Du Preez *et al* (2003) the following is recommended:

- **Level 1:** *Screening surveys* – Collection of a composite sample consisting of eight individuals at each site. Preferably three composite samples, each consisting of eight individual at 10 percent of the screening sites. The mean length (size) of the individuals of the composite sample and the mean length of individuals in all the composite samples must not exceed 10%.
- **Level 2:** *Intensive surveys, Phase I*. – Collection of five replicate composite samples, each consisting of eight individuals. As this would not be possible for some of the rivers (due to small fish populations) in South Africa, statistical procedures (as indicated) should be used to evaluate the statistical significance of the discussion.
- **Level 3:** *Intensive surveys, Phase II*. – The same recommendations as for Level 2 surveys.

When individual fish samples are collected for use in chemical contaminant surveys (which are not generally used) it is recommended that 25 individuals of a specific species in the required size range are collected.

Sampling time and sampling frequency.

When considering the time of sampling environmental considerations (for example high rainfall, floods, water temperature, etc.), spawning period (may affect respiration rates, lipid content of tissue, feeding habits of fish) and peak harvest time should be considered. The US EPA (1995a) suggests that the sampling period should not occur during the spawning season as well as one month prior to and after spawning. No general consensus of the frequency of sampling exists. The US EPA (1995a) recommends that if resources are available screening should be biannual for water-bodies where commercial, recreational or subsistence fishing is practiced. However, these water-bodies should be screened at least once every 5 years. Heath (1999) concluded that logistically it would not be possible to perform biannual surveys and suggested that surveys be undertaken every 3 to 5 years. However, the sampling frequency should be determined by the potential severity of the predicted health risk and the importance of the water-body to recreational, subsistence and commercial fishing.

The following sampling time and frequency of sampling are recommended for South African water-bodies:

- **Level 1:** *Screening surveys* – Fish should be collected from March to May and from September to October. The frequency of screening should be linked to the importance of the water-body to recreational, subsistence and commercial fishing. The frequency should be three years but definitely every five years. However, if potentially high health risks are predicted and the fish population is intensively fished then annual screening of the specific water-body should be undertaken.
- **Level 2:** *Intensive surveys, Phase I.* – The screening period must be the same as for the Level 1 surveys. The survey should be undertaken within one year of the Level 1 (screening survey).
- **Level 3**: *Intensive surveys, Phase II*: – The general guidelines for a Level 2 survey should be followed. In many cases it would be feasible and more cost effective to combine Level 2 and Level 3 surveys.

STEP 4: FIELD COLLECTION

Fish sampling equipment

Various fishing methods are available to collect freshwater fish. The methods employed will depend on the specific water-body (for example river or lake), the manpower and the equipment available. Although each of the methods has advantages and disadvantages the selected fishing methods must be able to capture a representative fish sample of the selected fish specie. In South Africa gill nets, seine nets, electro-fishing, line and hook and purchasing of fish can be used to obtain fish samples for the different survey levels (Table 12). It is recommended Table 12 can be used as a guide for fish sampling methods for South African water-bodies.

Species identification and sorting

Species should be identified as soon as they are captured. Experienced personnel using the appropriate taxonomic keys must perform specie identification. The publications by Jubb (1967), le Roux & Steyn (1968), Pienaar (1978) and Skelton (2001) should be used to identify South African freshwater fish species. If a fish species cannot be identified a fish

taxonomist, for example from the J.L.B. Smith Institute of Ichthyology in Grahamstown, South Africa, should be contacted to assist with the identification.

Table 12. Fish sampling methods that can be used in South Africa (adapted from US EPA, 1995a)

EQUIPMENT	AREA OF USE	ADVANTAGES	DISADVANTAGES
Gill nets	Lakes, reservoirs and rivers	• Effective to collect pelagic fish species. • Easy to operate and fishing effect reduced • Selective catches due to the use of different mesh sizes.	• Bottom dwelling fish or fish with restricted movement pattern not effectively captured. • Nets damaged and tangled by large species of fish. • Can kill captured fish, which will undergo physiological changes if not frequently removed. • Captured fish eaten by other animals e.g. crabs and otters. • Hazard to water sport • Ineffective in fast-flowing water or river with debris
Seine nets	Lakes and shallow rivers	• Relatively inexpensive and easy to operate. • Selective catches due to the use of different mesh sizes. • Fish not needed can be returned unharmed.	• Not effective in deep water, substrates with irregular contours and rocky bottoms. • Manpower requirements may be limiting.
Electro fishing	Shallow rivers and lakes	• Efficient nonselective method. • Minimal damage to fish. • Adaptable to a number of conditions, for example wading and from a boat.	• Non-selective as it stuns most fish • Not effective in deep, fast-flowing rivers. • Requires extensive operator training. • Dangerous if not used correctly.
Hook and line	Lakes, reservoirs and rivers	• Most selective method. • Equipment not too expensive. • Large number of personnel not required.	• Inefficient. • Not dependable.
Purchasing specimens from fishermen	Only in cases where selected species are harvested commercially or by subsistence fishing	• Most cost-effective and efficient method to obtain commercially valuable species	• Limited use as commercially harvested areas may not include the selected sites. • Specimens not collected and stored according to monitoring protocol.

After capture and depending on the circumstances the initially selected fish species can be transferred to a holding tank filled continuously with water from the site (du Preez *et al*. 1997). Fish should, however, not be kept in the holding tank for more than three hours. The specific number of selected fish species must be collected to make up the composite samples. Fish that do not meet the required size and are not from the selected species should be returned to the water-body. All fish with damaged fins or skin must also be discarded (US EPA, 1995a).

Individuals of the identified selected species should be rinsed in ambient water to remove any foreign material from their body surface. A sharp blow on the skull with a clean wooden club or metal rod designed for this purpose (to prevent contamination) should stun large fish. Small fish may be placed on ice to kill them humanely. Stunned fish are then grouped and placed in clean holding trays to prevent contamination. Care should be taken not to stun too many fish at a time in the field, especially during summer, as rate of decay is rapid.

Size measurements

Individual fish of the selected species should be measured to determine the total body length (mm). Total body length is defined as the length from the tip of the mouth to the tip of the largest caudal fin ray and should be measured as shown in Figure 4. Other external and internal features of a typical bone fish are shown in Figure1.4 and Figure 5.

Fish health observation

As a rapid and inexpensive alternative to more sophisticated approaches for evaluation of fish health and condition, Goede and Barton (1990) developed a field necropsy method. This method provides a health profile of fish based on the percentages of anomalies observed in the tissue and organs of individuals sampled from a population (Robinson, 1996; Groenewald & du Preez, 1998, Avenant- Oldewage 2001). A literature review of fish health assessment techniques is attached as Appendix A.

Even though the necropsy method provides a health status profile of a fish population, there is no quantitative basis of comparing statistically the entire index with all its variables to another population sample either in time or space. The Health Assessment Index (HAI) developed by Adams *et al.* (1993) is intended to minimise these limitations of the necropsy method by rendering it quantitative for statistical analysis and comparisons among data sets. For the HAI to have a statistical basis, all variables within the index must be assigned a numerical value. Adams *et al*. (1993) thus assign a numerical value of condition to each variable based on the original necropsy classification of criteria of Goede and Barton (1990) within the HAI. Avenant-Oldewage (2001) adapted and refined the HAI (See Appendix B) and applied it at various system in South Africa. In most cases it would not be possible to combine the collection of fish for chemical contaminant investigation with the Health Assessment Index (HAI) protocol developed by Adams or proposed by Avenant-Oldewage (2001). This can be attributed to the following:

- It is time-consuming to complete the HAI as it consists of at least eighteen variables.
- Some of the variables require inspection of the internal organs (Figure 5) for which the fish must be cut open. This should not be done if the fish must be stored.
- Skilled personnel are required to perform the evaluation.
- Specialised equipment is required.

However, it is important to note gross morphological abnormalities and the body surface parasite load of the fish captured. The health of the selected specie is thus evaluated according to A Fish Health Assessment Index (FHAI, Appendix B) consisting of the following variables:

- Condition of skin.
- Condition of fins.
- Condition of eyes.
- Condition of opercula.
- Condition of gills.
- Number of ectoparasites.

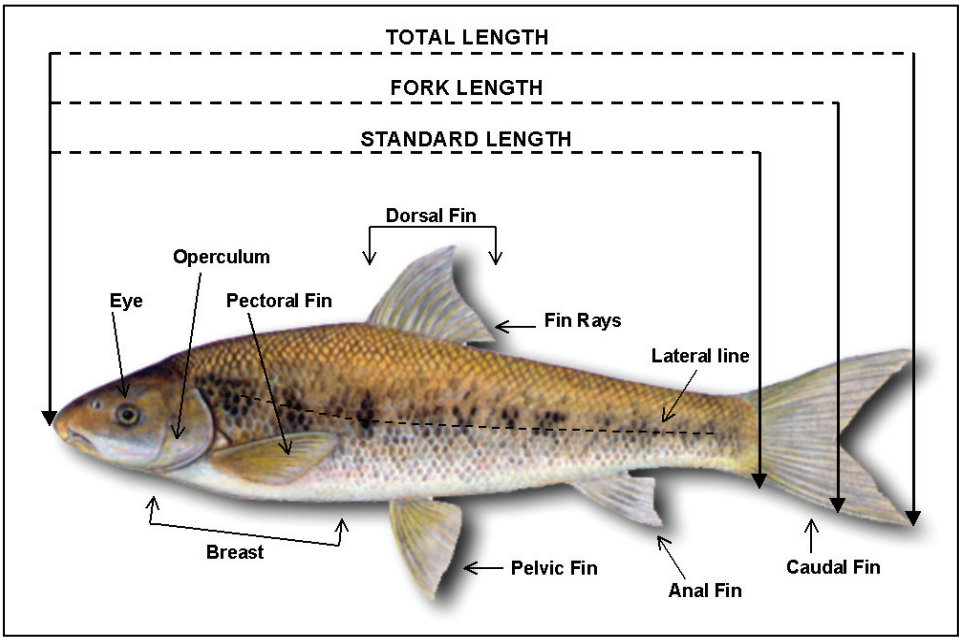


Figure 4. External features and size measurements of a freshwater bone fish (adapted from Skelton, 2001)

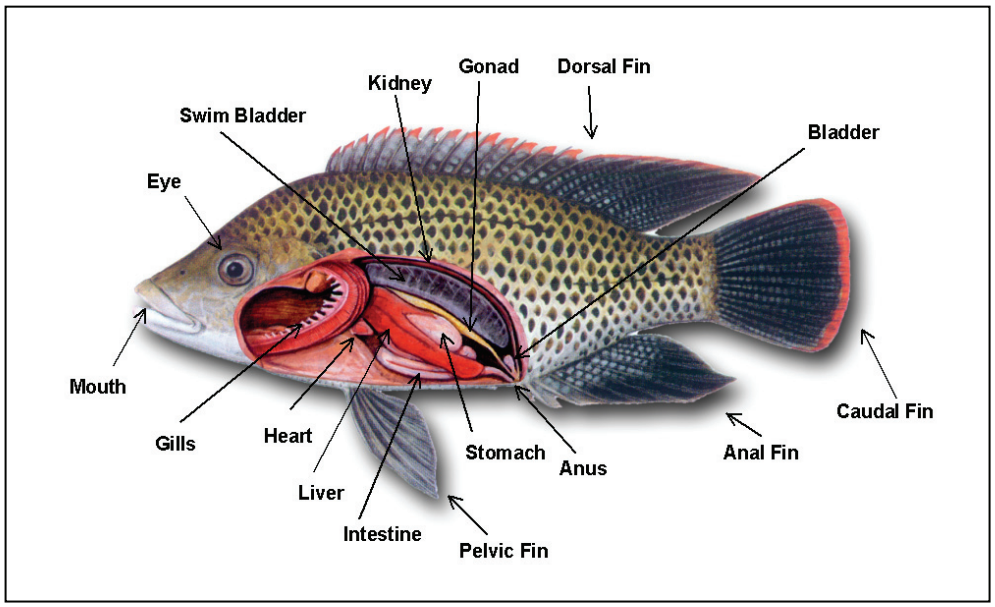


Figure 5. External features and internal organs of a freshwater bone fish (adapted from Skelton, 2001)

The description of these variables and the associated sources are summarised in Table 13. The health of the fish can then be calculated as follows:

$$\mathbf{FHAI_{(fish)} = S+F+E+O+G+P}$$

where :

- S = skin, F = fins, E = eyes, O = opercula, G = gills, P = external parasites

The FHAI for a specific species is calculated as follow:

$$\mathbf{FHAI_{(Species\ A)} = median\ (FHAI_{(fish\ 1,\ species\ A)}, FHAI_{(fish\ 2,\ species\ A)}, \ldots\ldots FHAI_{(fish\ n,\ species\ A)})}.$$

where:

- n = the number of fish sampled of a specific species.

The FHAI for a site is calculated as follows:

$$\mathbf{FHAI_{(site)} = median\ (FHAI_{(species\ a)}, FHAI_{(species\ B)}\ldots\ldots FHAI_{(species\ n)})}.$$

where:

- n = the number of species sampled at a site.

It is therefore recommended that the health of the fish is evaluated by using the HAI as described by Avenant-Oldewage (See Appendix A; Avenant-Oldewage 2001) or the FHAI procedure described above. The selection will depend on the specific requirements and available recourses

Sample packaging and preservation

Each fish should be individually wrapped in extra heavy aluminium foil and placed in a waterproof plastic bag (Table 14). However, aluminium foil should not be used for long term storage of the sample if it would be used for metal analysis. Spines of the fish must be sheared to reduce the risk of puncturing the aluminium foil. Individual fish of a composite sample should be sealed in a waterproof plastic bag and then packed together in a large plastic bag. The identification tag must be sealed waterproof and attached to the individual samples. Once packed the samples must be cooled immediately (US EPA, 1995a).

Depending on the transport time the samples can be kept on wet ice packets or frozen on dry ice (Table 14). On arrival at the analytical facility the sample should be inspected to ensure it was preserved during transportation. After inspection the fish must be processed or stored frozen as indicated in Table 15.

Table 13. Fish Health Assessment Index (FHAI) variables and assigned values (Appendix B). Based on the necropsy system of Adams *et al.* (1993) and Robinson (1996)

VARIABLES	VARIABLE CONDITION		SCORE VALUE FOR FHAI
Skin	• Normal, no aberrations		0
	• Mild skin aberrations		10
	• Moderate skin aberrations		20
	• Severe skin aberrations		30
Fins	• No active erosion or previous erosion healed over		0
	• Mild active erosion with no bleeding		10
	• Severe active erosion with hemorrhage/secondary infection		20
Eyes	• Normal	• No aberrations evident (good "clear" eyes)	0
	• Exopthalmia	• Swollen, protruding eye	30
	• Hemorrhagic	• Bleeding in the eye	30
	• Blind		30
	• Missing	• Eye missing from the fish	30
	• Other	• Any manifestations which do not "fit" the above	30
Opercules	• No shortening	• Normal	0
	• Mild shortening		10
	• Severe shortening		20
Gills	• Normal	• No apparent aberration in gills	0
	• Frayed	• Erosion of tips of gill lamellae: "ragged" look	30
	• Clubbed	• Swelling of the tips of the gill lamellae	30
	• Marginate	• Gill with a light discoloured margin along the distal end or tips of the lamellae of filament	30
	• Pale	• Gills are definitely very light in colour	30
	• Other	• Any observation which does not fit above	30
Ectoparasites	• No parasites observed		0
	• 1 – 10 parasites		10
	• 11 – 20 parasites		20
	• > 20 parasites		30

Table 14. Recommended preservation of fish samples from time of collection to delivery at the laboratory (adapted from the US EPA, 1995a)

SAMPLE TYPE	NUMBER PER COMPOSITE	CONTAINER	PRESERVATION	MAXIMUM TRANSPORT TIME
Whole fish to be filleted and/or whole fish	8	Each fish wrapped in heavy-duty aluminium foil and placed in a waterproof plastic bag.	Cool on wet ice or ice packets Or Freeze on dry ice only if transport time is more than 24 hours	24 Hours 48 Hours

Documentation and document control

Thorough documentation will ensure that the correct data is collected and that all the field sample collection and handling information is available for interpretation (US EPA, 1995; du Preez 1999). The following documents are suggested (US EPA, 1995a; du Preez, 1999; du Preez *et al.* 2000):

- Field sample request form (Table 16)
- Field sampling record form (Table 17)
- Fish Health Assessment index Form (Table 18)
- Fish sample Identification label (Table 19)
- Chain of custody label (Table 20)
- Chain of custody record form (Table 21)

The control of documentation is vital and if an accredited the laboratory is used, the document control requirements as prescribed by the International Standard ISO/IEC 17025: General requirements for the competency of testing laboratories (ISO/IEC, 1999) must be followed. The document control requirements as described by the International Standard SABS ISO 14001: 1996: Environmental management systems – Specification with guidance for use (SABS ISO, 1996) should also give guidance to the implementation of document control measures. It is recommended that the project leader or designated person design specific forms to ensure proper documentation. Furthermore, document control requirements as described in the above mention international standards should be implemented.

Table 15. Summary of recommendations for container materials, equipment, washing material, preservation and holding times per fish tissue from sample processing to analysis (adapted from US EPA, 1995a)

ANALYTE	MATRIX	EQUIPMENT	WASHING MATERIAL	SAMPLE CONTAINER	STORAGE	
					Frozen	Holding time
Mercury	‣ Fillets and homogenates.	‣ Quartz or PTFE or polypropylene or polyethylene or Borosilisate glass. ‣ Dissection knives: Titanium blades and PTFE handles. ‣ Dissection boards: glass or PTFE covered with aluminium foil. ‣ Bench liners: borosilicate glass. ‣ Instruments: quarts or titanium.	‣ Detergent solution e.g. contrad. ‣ Soaked in 50% HNO_3 for 12 to 24 hrs. ‣ Rinsed with metal-free distilled deionised water.	‣ Plastic or borosilicate glass or quartz or PTFE.	Freeze at $\leq$ -20°C.	28 days
Other Metals	‣ Fillets and homogenates.	‣ Quartz or PTFE or polypropylene or polyethylene or Borosilisate glass. ‣ Dissection knives: Titanium blades and PTFE handles. ‣ Dissection boards: glass or PTFE covered with aluminium foil. ‣ Bench liners: borosilicate glass. ‣ Instruments: quarts or titanium.	‣ Detergent solution e.g. contrad. ‣ soaked in 50% HNO_3 for 12 to 24 hrs. ‣ Rinsed with metal-free distilled deionised water.	‣ Plastic or borosilicate glass or quartz or PTFE.	Freeze at $\leq$ -20°C.	6 months
Organics	‣ Fillets and homogenates	‣ Stainless steel or anodized aluminium or borosilicate glass or PTFE or ceramic or quartz. ‣ Dissection knives: Titanium blades and PTFE handles. ‣ Dissection boards: glass or PTFE covered with aluminium foil. ‣ Bench liners: borosilicate glass. ‣ Instruments: Stainless steel or quarts or titanium.	‣ Detergent solution e.g. contrad. ‣ Soaked in pesticide grade isopropanol or acetone. ‣ Rinsed with organic-free distilled deionised water.	‣ PTFE or borosilicate glass or quartz or aluminium foil.	Freeze at $\leq$ -20°C.	1 year

Table 15. (Continued).

ANALYTE	MATRIX	EQUIPMENT	WASHING MATERIAL	SAMPLE CONTAINER	STORAGE	
					Frozen	Holding time
Metals and Organics	‣ Fillets and homogenates	‣ Borosilicate glass or PTFE or ceramic or quartz. ‣ Dissection knives: Titanium blades and PTFE handles. ‣ Dissection boards: glass or PTFE covered with aluminium foil. ‣ Bench liners: borosilicate glass ‣ Instruments: quarts or titanium .	‣ Detergent solution e.g. contrad. ‣ Soaked in 50% HNO_3 for 12 to 24 hrs. ‣ Rinsed with metal and organic-free distilled deionised water.	‣ Quarts or borosilicate glass or PTFE.	Freeze at $\leq$ -20°C.	‣ 28 days for mercury. ‣ 6 months for other metals. ‣ 1 year for organics.
Lipids	‣ Fillets and homogenates	‣ Borosilicate glass or PTFE or ceramic or quartz. ‣ Dissection knives: Titanium blades and PTFE handles. ‣ Dissection boards: glass or PTFE covered with aluminium foil. ‣ Bench liners: borosilicate glass ‣ Instruments: quarts or titanium .	‣ Detergent solution e.g. contrad. ‣ Soaked in 50% HNO_3 for 12 to 24 hrs. ‣ Rinsed with metal and organic-free distilled deionised water.	‣ Plastic or borosilicate glass or quartz or PTFE.	Freeze at $\leq$ -20°C.	1 year

Table 16. An example for a fish sample request form (adapted from the US EPA, 1995a)

FISH SAMPLE REQUEST FORM FOR FISH CONTAMINANT SURVEY

Project Objective
- ☐ Level 1 Survey
- ☐ Level 2 Survey
- ☐ Level 3 Survey

Sample Type
- ☐ Fish Fillets only
- ☐ Whole fish or portions other than fillet *(Specify tissues used if other than whole)*

_____

Selected Contaminants
- ☐ All target contaminants
- ☐ Additional contaminants *(Specify)*

_____

- ☐ Fish Fillets only
- ☐ Whole fish or portions other than fillet *(Specify tissues used if other than whole)*

_____

- ☐ Contaminants exceeding screening study SCs ***(Specify)***

_____

_____

INSTRUCTIONS TO SAMPLE COLLECTION TEAM

Project Number: _____ **Site (Name/Number):** _____

Province: _____ **GPS Coordinates:** _____

Selected Species: **Alternate Species:** ***(in order of preference)***

_____ _____

_____ _____

_____ _____

_____ _____

Proposed Sampling Dates: _____

Proposed Sampling Method:

- ☐ Electro-fishing
- ☐ Gillnetting
- ☐ Seining
- ☐ Hook and Line
- ☐ Other *(Specify*)_____

Number of Sample Replicates: ☐ **No field replicates (1 composite sample only)**

☐ _____ field replicates
(Specify number for each selected species)

Number of Individuals per Composite: _____ Fish per composite

Table 17. An example for a field record form for fish contaminant surveys (adapted from the US EPA, 1995a)

FIELD RECORD FOR FISH CONTAMINANT SURVEY

Project Number:_____ **Sampling Date and Time:** _____
Survey Type: Level 1 survey □ Level 2 survey □ Level 3 survey □

SITE LOCATION

Site Name/Number:

Province: | **GPS Coordinates:**

Waterbody Name: _____ **Waterbody Type:** □ RIVER □ LAKE

Site Description: _____

Collection Method: _____

Collector Name *(print and sign)*: _____

Agency: _____ **Phone:** (_____) _____

Address:

FISH COLLECTED

Bottom Feeder – Species Name: _____

Composite Sample #: _____ **Number of individuals:** _____

Fish #	Length (mm)	Sex	Fish #	Length (mm)	Sex
001			005		
002			006		
003			007		
004			008		

Minimum size / Maximum size x 100 = _____ **> 75%** **Composite mean length** _____ **mm**

Notes (e.g., morphological anomalies):
_____

Predator – Species Name: _____

Composite Sample #: _____ **Number of Individuals:** _____

Fish #	Length (mm)	Sex	Fish #	Length (mm)	Sex
001			005		
002			006		
003			007		
004			008		

Minimum size / Maximum size x 100 = _____ **> 75%** **Composite mean length** _____ **mm**

Notes (e.g., morphological anomalies):
_____

Table 18. An example for a fish health record form

FISH HEALTH ASSESSMENT RECORD

Project Number: _____ **Sampling Date and Time:** _____

STUDY PHASE: Level 1 Survey ☐ Level 2 Survey ☐ Level 3 Survey ☐

SITE LOCATION

Site Name/Number: _____

Province: _____ **GPS Coordinates**:_____

Waterbody Name: _____ **Waterbody Type:** _____

Sample Type (bottom feeder, predator) _____

Species Name: _____

Composite Sample #: _____ **Replicate No**: _____ **No. of Individuals:** _____

HEALTH SCORE	FISH #							
	001	002	003	004	005	006	007	008
Skin								
Fin								
Eye								
Opercula								
Gill								
Ectoparasite								
TOTAL SCORE								

Notes: _____

_____

Table 19. An example for a fish sample identification label (adapted from the US EPA, 1995a)

FISH SPECIES NAME OR CODE	**SAMPLE TYPE**
Total Length or Other *(specify)* (cm):	**Sampling Site *(name/number)***
Fish Number □ □ □ □ □ - □ □ □	**Sampling Date (day/month/year)** **Time (24-h clock)**

Table 20. An example for a chain-of-custody label for fish contaminant surveys (adapted from the US EPA, 1995a)

PROJECT NUMBER	**COLLECTING AGENCY (NAME, ADDRESS, PHONE)**			
Sampling Site (name and/or ID number)	**Sampler (name and signature)**			
Composite Number or Fish Number(s)	**Chemical Analyses** □ All selected analyses □ Others (specify) _____ _____		**Survey Type** Level 1 □	Level 2 □
Sampling Date (d/m/yr) **Time (24-hr clock)**				Level 3 □
Species Name or Code	**Processing**		**Type of Ice**	
	Whole Body	Dissection	Wet	Dry
Comments:				

Table 21. An example for a chain-of-custody record form for fish contaminant surveys (adapted from the US EPA, 1995a)

CHAIN OF CUSTODY RECORD

Project No.	**Collecting Agency (name, address, phone)**					**Sampling Date**	**Selected Contaminants**	**Specific Contaminants**	**Chemical Analyses**
Samplers (print and sign)						**Container ____ of ____**			
Composite Number	**Specimen Number**	**Sampling Time**	**Survey type** Level 1	Level 2	Level 3	**Sampling Site (name/number)**			**Comments**

Delivery Shipment Record		**Deliver/Posted to: (name, address and phone)**				**Date/Time Send:**	
Delivery Method ; □ Hand carry □ Mail							
Relinquished by: ***(signature)***	**Date**	**Time**	**Received by:** ***(signature)***	**Relinquished by:** ***(signature)***	**Date**	**Time**	**Received by** ***(signature)***
Relinquished by: ***(signature)***	**Date**	**Time**	**Received for Lab. by:** ***(signature)***	**Date**	**Time**	**Remarks:**	

Laboratory Custody:			
Released Name/Date	**Received Name/Date**	**Purpose**	**Location**

STEP 5: LABORATORY SAMPLE PROCESSING AND ANALYSIS

Fish sample processing

Sample processing is an important step in the process of determining the concentrations of analytes in the fish population and must therefore be performed by competent personnel. The different sample processing activities in the laboratory that relate to the preparation of fish fillet composite homogenate samples for analyses could be performed stepwise. Data obtained during the different processing steps should be recorded to ensure traceable records (US EPA, 1995a). An example of a sample processing record is shown in Table 22.

Laboratory conditions, instrumentation and sample storage requirements

Sample processing should be performed under laboratory conditions that would minimize the risk of contamination. It is preferable not to process samples in the field (US EPA, 1995a). If samples are processed in the field a specific area away from any fuel fumes or other possible airborne contaminants should be allocated. The use of a mobile field laboratory or working on a portable dissection table with an enclosed hood is advisable.

Potential sources of sample contamination include dust (airborne and surface), instruments, utensils, work surfaces and containers that may come in contact with the samples. It is important to note that polypropylene and polyethylene (plastic) surfaces, implements, gloves and containers are a potential source of contamination of organic analytes and should not be used when samples are used for organic analysis. Instruments, work surfaces and containers used during processing of samples must be of materials that can be cleaned easily and that are not themselves sources of contamination. Furthermore to prevent cross-contamination all equipment used in sample processing should be cleaned before each sample is prepared (US EPA, 1995a). The suggested sample processing equipment, container materials and holding time for fish are summarised in Table 15.

Sample inspection

The individual fish received for processing should be inspected carefully to ensure that they were adequately preserved during transportation. Fish not suitable for further processing and/or analysis should be discarded and recorded on the sample processing record.

Sample weighing

The wet mass (to the nearest gram) of each fish should be determined. All weighing should be done on balances with the required accuracy and precision and calibrated by following good laboratory practice (GLP) procedures. Excess ice should be wiped from the fish body surface. Liquid from thawed whole fish samples may be from the body cavity and gut and not necessarily from the tissue to be filleted. As a precautionary approach all liquid should be kept as part of the sample (US EPA, 1995a). Nevertheless, fish should be weighed and filleted quickly to minimise the formation of liquid during thawing.

Age and sex determination

Fish scales, otoliths or pectoral fin spines (for example from catfish) can be removed for age determination. Five to ten scales can be removed from the area between the dorsal fin and

the lateral line behind the pectoral fin. The scales, spines or otoliths may be stored in small plastic bags or envelopes, which are clearly marked for cross-reference. Scales can be washed in soapy water and mounted between glass slides, whereafter the growth rings can be counted using a microfiche projector. Thin sections of spines and otoliths can be cut and the growth rings counted under a compound microscope (van der Waal & Schoonbee, 1975; Everhart *et al*. 1976; Heath, 1999).

Table 22. An example of a fish sample processing record for fish contaminant surveys (adapted from the US EPA, 1995a)

FISH SAMPLE PROCESSING RECORD FOR FISH CONTAMINANT SURVEYS
FISH FILLET COMPOSITES

Project Number: _____ **Sampling Date and Time:** _____

SURVEY TYPE: Level 1 Survey ☐ Level 2 Survey ☐ Level 3 Survey ☐

SITE LOCATION

Site Name/Number:_____

Province:_____ **GPS Coordinates:** _____

Waterbody Name: _____ **Waterbody Type:** _____

Sample Type (bottom feeder, predator) _____ **Species Name:** _____

Composite Sample #: _____ **Replicate Number** : _____

Number of Individuals: _____

					First Fillet (F1) Or Combined Fillets (C)			**Second Fillet (F2)**		
Fish #	**Mass (g)**	**Scales or Otoliths Removed (3)**	**Sex (M,F)**	**Dissection Performed (3)**	**Mass (g)**	**Homo-genate Prepared (3)**	**Mass of Homo-genate for Composite (g)**	**Mass (g)**	**Homo-genate Prepared (3)**	**Mass of Homo-genate for Composite (g)**
001										
002										
003										
004										
005										
006										
007										
008										
Ana -lyst										
Date										

Total Composite Mass (g): (F1 or C) _____ **(F2)** _____

Notes:

_____

If the sex of the species cannot be determined by external inspection, the body cavity should be cut open (incision on ventral body surface from immediately anterior to the anus to immediately posterior of the pelvic fins) to inspect the gonads (Figure 5). The gender of the fish and stage of reproduction can then be determined by using the classification system of gonad development (Table 23) as described by Olatunde (1978). The age determination of individual fish is optional but the size of the fish, a description of the reproductive stage and sex determination should be performed.

Fish health observation

The Fish Health Assessment Index should preferably be performed during the field collection stage. Only the FHAI assessment can be performed, but it would not be possible to determine their ectoparasite load as these parasites usually detach themselves from dead fish. For South Africa it is recommended that the fish health observations are made directly after capture.

Scaling and skinning of fish

Fish with scales should be scaled and any slime removed before filleting. Catfish, *Clarias gariepinus* and other scaleless fish species should be skinned prior to filleting. After removing the scales and slime or the skin, the outside of the fish should be rinsed with contaminant-free distilled water and placed on a clean dissection board for filleting. Fish should not be allowed to thaw completely as it is best to fillet fish while ice crystals are still present in the muscle tissue. The procedure to remove the scales or skin and fillets from the fish are illustrated and briefly described in Figure 6. The belly flap is included in the fillet as well as any dark tissue found with the white tissue. Skeletal bones that may be present should, however, be removed (US EPA, 1995a). The belly of the fish is then dissected open (cutting from the anus in the direction of the head) to remove other tissue (fat, liver and gonads) individually. Puncturing of internal organs must be avoided, as the material released from the internal organs will contaminate the fillets and the other tissues. After removing all of the fillets, fat, liver and gonads they are weighed after which they are processed further or stored (Table 15).

Preparation of individual and composite homogenates

The fillets and other tissue from individual fish must individuality be ground and homogenised prior to analysis. This would ensure even distribution of contaminants throughout the sample and enhance the extraction and digestion of the tissue. Grinding should continue until the sample appears homogeneous. The sample is then divided into quarters. The opposite quarters are mixed together and then the two halves mixed again. This process of grinding, quarterly and mixing should be repeated at least twice (US EPA, 1995a). Thereafter the individual homogenates are either processed further to prepare composite homogenates or stored separately as indicated in Table 15.

Composite homogenates are prepared from individual homogenates of equal mass. It is important to prepare composite homogenates from the same type of individual homogenates (either single fillet or tissue of combined fillet or tissue). Each composite homogenate is blended as previously described for individual homogenates. After preparation of the composite homogenates they may be processed for analysis or stored as described in Table 15. The portion of the individual homogenate sample that is not used may be stored as a correctly labeled “Archive” sample and re-used if required (US EPA, 1995a). The various

steps of the preparation of fish fillet and other tissue homogenates are summarised in Figure 7.

Distribution of samples

In some instances samples must be distributed to different laboratories for specific analyte analyses. For this purpose aliquots of specific weight (to the nearest 0.1 g) as required by the laboratory are prepared. It is essential that the sample is handled (that is, stored, transported, etc.) as previously described to prevent deterioration or contamination of the sample (US EPA, 1995). Furthermore, detailed traceable records during the preparation of the aliquots (Table 24) and the transfer of the aliquots to a specific laboratory (Table 25) must be kept.

Selection of analytical laboratory

It is a prerequisite that the selected analytical laboratory would perform the analysis using internationally accepted analytical methods and has well-documented quality assurance and quality control systems in place. Preferably the analysis should be performed by a laboratory which has been accredited under the international standard ISO/IEC 17025 (ISO/IEC, 1999).

Table 23. Criteria for the classification of fish gonad development (Olatunde, 1978)

G1 STAGE	CHARACTERISTIC
0. Inactive (I)	Small gonads and close to the vertebral column. Gonads transparent and gray.
1. Inactive-Action (IA)	Testes and ovaries translucent, gray-red. Single eggs just visible to the naked eye. Gonads extending most of the length of the ventral cavity.
2. Active (A)	Eggs visible to the naked eye. Gonads reddish with blood capillaries, filling 🗎 – 🗁 of the ventral cavity.
3. Active-Ripe (AR)	Ovaries orange-red. (Not *Clarias gariepinus*-gonads remain gray). Testes white with red blood vessels. No milt-drops appear under pressure. Eggs opaque.
4. Ripe ®	Sexual products mature. Testes exude milt when pressure exerted. Eggs spherical.
5. Ripe-Running (RR)	Eggs and milt running with slight pressure.
6. Spent (S)	Gonads have the appearance of deflated sacs, reddish colour. Occasional residual eggs and some milt.

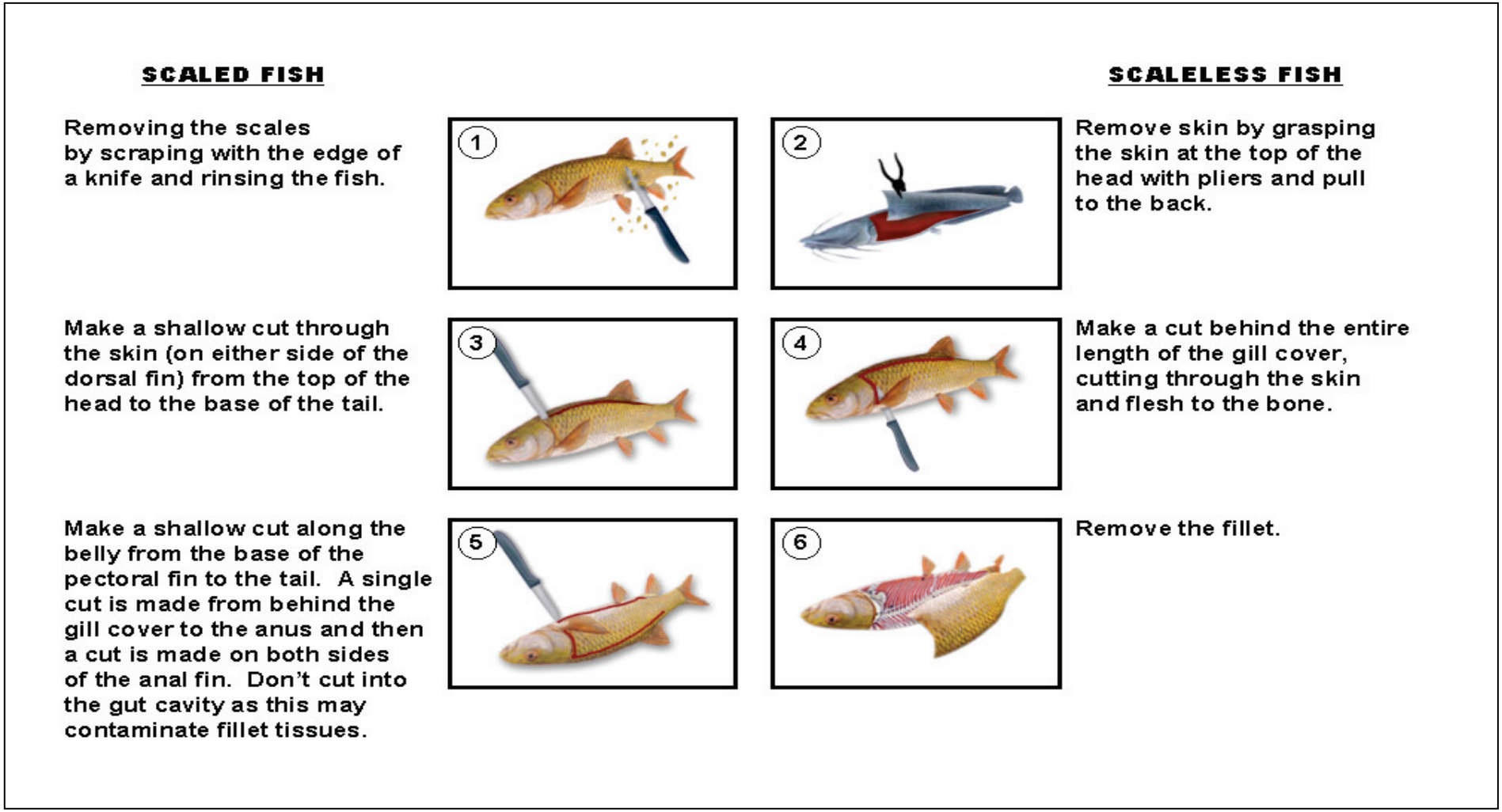


Figure 6: Filleting of freshwater fish for chemical contaminant analyses in fillets (adapted from the US EPA, 1995a)

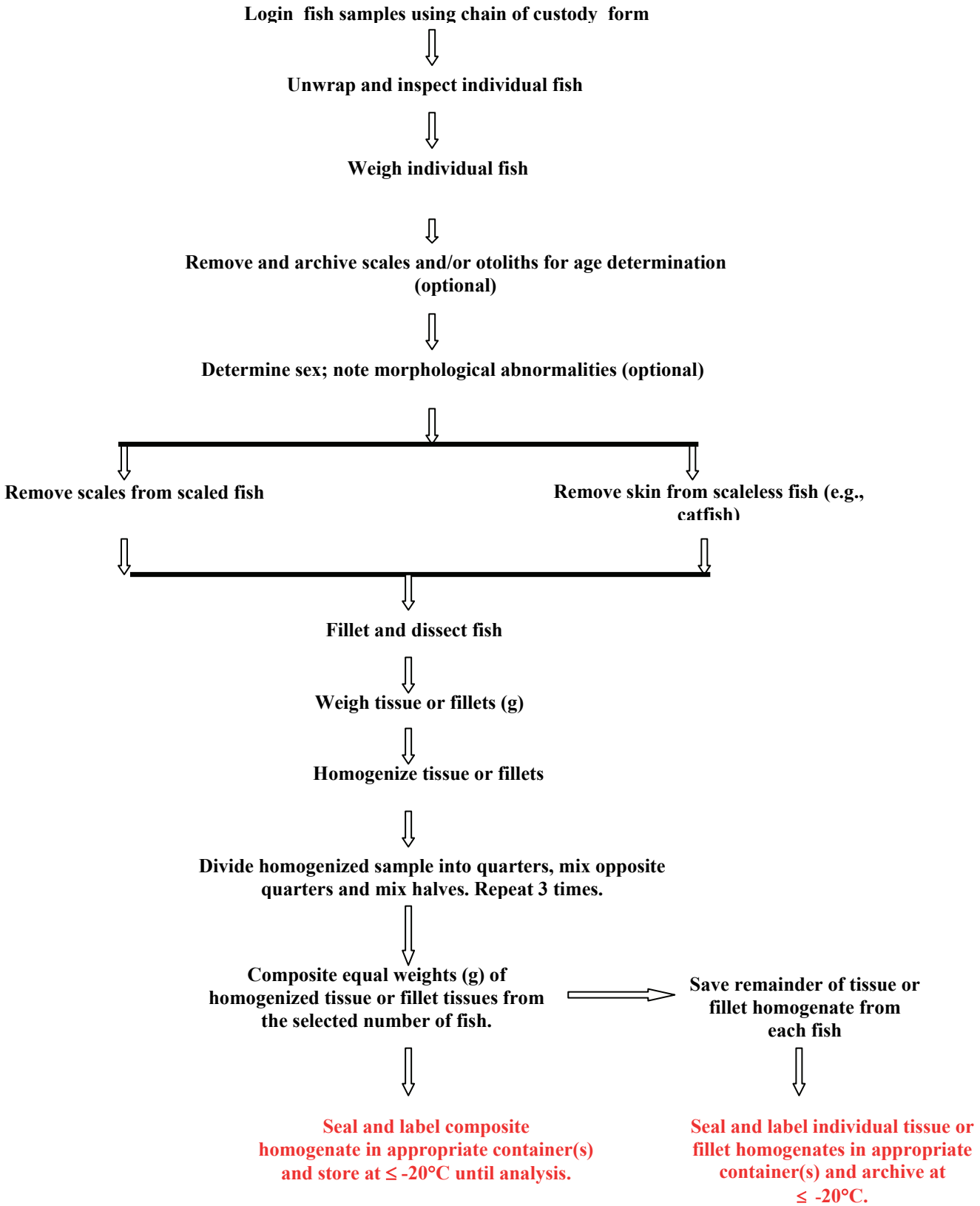


Figure 7: Summary of the steps in the laboratory preparation of fish fillet or other tissue composite homogenate samples (adapted from the US EPA, 1995a)

Table 24. An example of a fish chemical contaminant survey aliquot sample record (adapted from the US EPA, 1995a)

FISH CONTAMINANT SURVEY
ALIQUOT SAMPLE RECORD

Aliquot prepared by:_____ **Date**:_____ **Time:**_____
(name)

Comments: _____

Samples from:
Project No.:_____ **Site #:**_____ □ Level 1 survey
□ Level 2 survey
□ Level 3 survey

Composite Sample ID	**Analyte Code ____**		**Analyte Code ____**		**Analyte Code ____**	
	Aliquot ID	**Aliquot Mass (g)**	**Aliquot ID**	**Aliquot Mass (g)**	**Aliquot ID**	**Aliquot Mass (g)**
Archive Location:	**Analyze for:** _____ _____ **Send to:** _____ _____		**Analyze for:** _____ _____ **Send to:** _____ _____		**Analyze for:** _____ _____ **Send to:** _____ _____	

Level 1 Survey: Screening Survey:
Level 2 Survey: Intensive Survey, Phase I:
Level 3 Survey: Intensive Survey, Phase II:

Comments:----------

Table 25. An example of a fish chemical contaminant survey sample transfer record adapted from the US EPA, 1995a)

FISH CONTAMINANT SURVEY
SAMPLE TRANSFER RECORD

Date : _____ _____ _____ Time _____ : _____ (24-h clock)
DD MM YY H M

Released by: _____
(*name*)

At: _____
(location)

Transport Method: _____

Transport Destination: _____

Date: _____ _____ _____ Time _____ : _____ (24-h clock)
DD MM YY H M

Received by: _____
(name)

At: _____
(location)

Comments: _____

_____

Study Type: ☐ Level 1 survey – Analyze for: ☐ Trace metals ☐ Organics ☐ Lipid

☐ Level 2 survey ☐ Level 3 survey – Analyze for *(specify)* _____

Sample Identifications:

Laboratory Chain of Custody

Relinquished by	Received by	Purpose	Location

In internationally accepted analytical methods and has well-documented quality assurance and quality control systems in place. Preferably the analysis should be performed by a laboratory which has been accredited under the international standard ISO/IEC 17025 (ISO/IEC, 1999). In South Africa SANAS (South African National Accreditation System) is the body responsible for the accreditation of analytical laboratories. The laboratories of the Agricultural Research Council (Private Bag X 313, Pretoria, 0001, South Africa), the Council for Scientific and Industrial Research (Environmentek, P O Box 35 Pretoria, 0001, South Africa), Institute of Water Quality Studies (Private Bag X 134, Pretoria, 0001, South Africa) and the South African Bureau of Standards (Private Bag X 191, Pretoria, 0001, South Africa) could be approached to perform the analysis. This statement is not an endorsement of the specific laboratories.

Analytical methods

Since various analytical methods are available world-wide for the analysis of specific chemical contaminants it is advisable that the programme manager in collaboration with the laboratory chemist responsible for the analysis discuss the appropriate methods. The following criteria can be used to select analytical methods for the routine analysis of analytes and lipids:

- Scientific merit – Methods should be technically sound, be specific for the selected analytes of concern and based on validated analytical techniques that are widely accepted by chemists.
- Sensitivity – Methods detection and quantification limits should be sufficiently low to allow reliable quantification of the selected analytes of concern at and below selected screening concentrations.
- Data quality – The accuracy and precision should be adequate to ensure that analytical results are of acceptable quality to achieve program objectives.
- Cost-effectiveness – Financial requirements to obtain the required resources should be realistic and competitive (US EPA, 1995a).

If the laboratory has accredited methods in place it is advisable to compare them with the methods proposed by the United States Environmental Protection Agency (US EPA) of the United States of America (USA) for the use in chemical contaminant monitoring programs. Reference to these methods can be found in the US EPA (1995a) publication, the automated US EPA Environmental Monitoring Methods Index System (National Technical Information Services, 5285 Port Royal Road, Springfield, VA 22161, USA; Internet: EMMIUSER@USVA5.DYNCORP.COM) and other EPA internet websites (htpp://www.epa.gov/). The publications by Van Loon (1980) and Watling (1981) also provide guidance to analytical procedures. For South Africa it is recommended that where practical the established US EPA procedures should be followed.

Quality assurance and quality control

The analytical laboratory performing the analysis must have documented quality assurance and quality control systems in place. Documented standard operating procedures must be available and followed. The requirements as prescribed by the International Standard ISO/IEC 17025 (ISO/IEC, 1999) should be followed. Furthermore, guidance to general health and safety practices can be obtained from systems such as the International Safety Rating System (ISRS, 1994). Nevertheless, the analytical standard operating procedures must at least include (but are not limited to) the following:

- Scope and application.
- Method performance characteristics (accuracy, precision, method detection and quantification limits) for each analyte.
- Interferences.
- Equipment, supplies and materials.
- Sample preservation and handling procedures.
- Instrument calibration procedures.
- Samples preparation (i.e. extraction, digestion, cleanup) procedures.
- Sample analysis procedures.
- Quality control procedures.
- Corrective action procedures.
- Data reduction and analysis procedures (with example calculations).
- Record-keeping procedures (standard data forms, etc.).
- Safety procedures and/or cautionary notes.
- Disposal procedures.
- References (US EPA, 1995a).

The minimum quality assurance and quality control requirements for the analysis of chemical contaminant fish samples include initial demonstrations of laboratory capability and the routine analyses of appropriate quality assurance and quality control samples to demonstrate continued acceptable performance and to document data quality (US EPA, 1995a). Initial demonstration of laboratory capability should include:

- Instrument calibration.
- Documentation of detection and quantification limits.
- Documentation of accuracy and precision.
- Analysis of an accuracy-based performance evaluation sample provided by an external quality assurance program.

The laboratory should demonstrate on an ongoing basis the acceptability of performance and documentation of data quality by:

- Routine calibration and calibration checks.
- Routine assessment of accuracy and precision.
- Routine monitoring of interferences and contamination.
- Regular assessment of performance through participation in external quality assurance (interlaboratory comparison) exercises (US EPA, 1995a).

Various quality assurance and quality control samples are available for use by the chemist and include:

- For external calibration. – Calibration standards.
- For internal standard calibration. – Instrument internal standards.
- For calibration verification. – Calibration check standards.
- For method detection limit determination. – Spiked matrix samples.
- For accuracy and precision assessment. – Reference materials, laboratory control samples, matrix spikes, matrix spike replicates, laboratory replicates, analytical replicates and field replicates.
- For contaminant assessment. – Blanks (for field techniques, methods, processing, containers and equipment, reagents).
- For monitoring of method performance for organic analysis. – Surrogate spikes.

- For external quality assessment. – Accuracy based performance evaluation samples and split samples.

Detailed descriptions (definitions, specifications, frequencies of analyses, control limits, corrective actions) of these quality assurance and quality control samples are given in the US EPA (1995a) publication. The above-mentioned assurance and quality control information should give some guidance to the project manager and the chemist of the basic quality assurance and quality control requirements when performing chemical contaminant analysis. However, if required, additional method-specific quality assurance and quality control procedures should be followed to improve overall quality of analytical results.

In South Africa the accreditation of methods at laboratories ensures that quality control and quality assurance procedures are in place and routinely followed. It is therefore recommended that only accredited laboratories be used for chemical contaminant analysis**.**

STEP 6: ANALYSIS AND REPORTING OF RESULTS

Recording of results by the laboratory

The recording of results must be performed according to the standard operating procedures developed for the recording of results. The integrity of the results (for example, transfer of result checks, approval of results etc.) must be verifiable at all times. The following is recommended for the recording of results:

- An analytical result below the method detection limit (MDL), including an analytical result recorded as not detected (that is no observed response) should be assigned a value of half the method detection limit (MDL/2).
- An analytical result recorded between the method detection limit and the method quantification limit (MQL) should be assigned a value of the method detection limit plus half the difference between the method quantification and the method detection limit [MDL + (MQC – MDL/2)].
- An analytical result recorded at or above the method quantification limit should be recorded as such (US EPA, 1995a).

Analysis of results

Level 1: *Screening surveys* – The results obtained should be evaluated to determine which of the results is greater than or less than the screening concentration (SC). The procedure of evaluation should also be documented. When the recorded analyte concentration is below but close to the SC, the data on the performance of the laboratory and historic data on water, sediment and fish tissue contamination at the site should be evaluated before further samples are taken. However, if the data of these investigations indicates that further investigation should be undertaken, a Level 2 survey should be initiated. A Level 2 survey will also be undertaken for the analytes that exceed the screening concentrations to verify the level of contamination (US EPA, 1995a).

Level 2: *Intensive surveys, Phase I* and **Level 3**: *Intensive surveys Phase II* – The main objectives of the Level 2 and Level 3 surveys are to assess the magnitude and geographical extent of the contamination (special variation) in the various classes of the selected species, to define the geographical region where fish contamination concentrations exceed the

screening concentrations, to identify the geographical contaminant concentrations and assess the fish contaminate concentrations over time (temporal variations).

As part of achieving these objectives the appropriate statistical methodology must be applied to the data. It is strongly recommended that a statistician be consulted throughout the study. This would ensure that (i) the statistical requirements of the objective of the study are met, (ii) the appropriate statistical tests are performed on the data obtained and (iii) the data is evaluated to determine the need for additional sample collection, risk assessment and issuing of fish consumption advisories. A general statistical approach for comparing replicate chemical contaminant results between two and more groups is summarised in Figure 8 (US EPA, 1995a). When following this procedure it is important to note the following:

- For each type of test several options are available, each of which may be appropriate in specific cases.
- If the assumptions of the parametric tests are met, then non-parametric tests should not be used.
- Logarithmic transformation is not appropriate in all cases and should not be performed if the data is sampled from a normally distributed population.
- Non-parametric tests are often performed on ranks, thus transformed data.
- Multiple comparison tests, comparable to those used for parametric data sets, are not available for non-parametric data sets.
- Regression analysis may be applied to determine temporal trends in contaminant data.
- If the percentage of lipid in a tissue sample is correlated with the chemical contaminant concentration (usually nonpolar organics) the contaminant concentrations can be normalised to the lipid concentration before statistical interrogation of the data. This would in some instances improve the power of the statistical tests (US EPA, 1995a).
- Before statistical evaluations of data are performed a statistician should evaluate the data to specify which statistical tests are appropriate for the specific data set.

Data storage

Since there is no general co-ordination of bioaccumulation studies in South Africa it is essential that data is stored in a central database. Heath (1999) proposed that a national bioaccumulation programme database be established. The proposed database should have the following characteristics:

- Coordinated by Department of Water Affairs and Forestry (DWAF), although the provinces may undertake the surveys.
- Department of Water Affairs and Forestry should be custodian of the monitoring programme.
- Database should be open and seamless.
- Housed at a central and accessible institution, for example at the Institute for Water Quality Studies of the Department of Water Affairs and Forestry.
- Updated regularly.
- Data must be available free of charge.
- The Internet provides an ideal infrastructure for such a shared database systems.

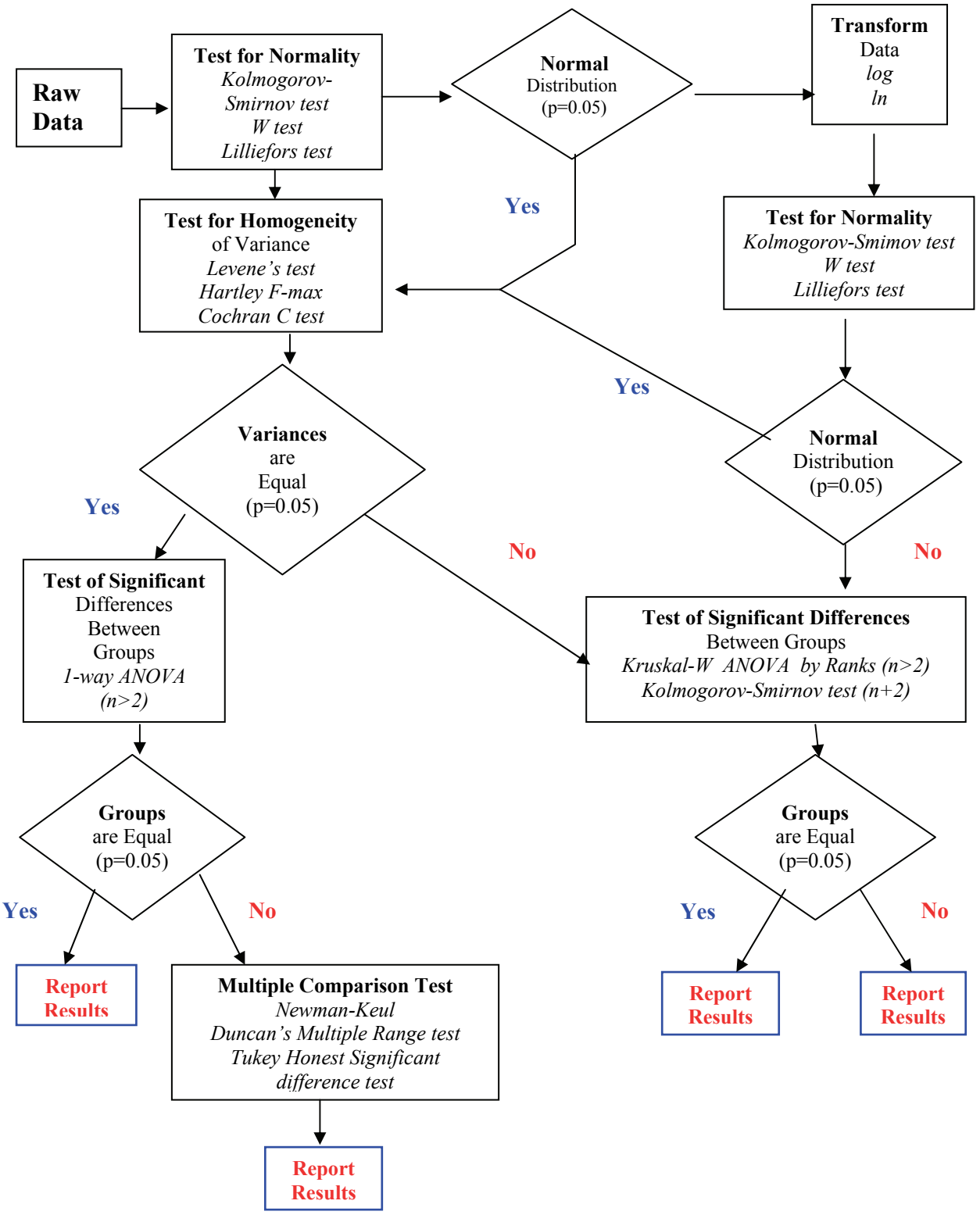


Figure 8. Statistical approach for testing of statistically significant differences between fish contaminant survey data sets (adapted from the US EPA, 1995)

It is recommended that the data obtained from the fish chemical contaminant surveys for the assessment of possible human health risks if the fish are consumed, forms part of this database. However, all the databases for fish chemical contaminant surveys including the

proposed (National Chemical Contaminant Database) should be structured in such a way that information and/or data in the following data fields can be entered:

- Study identification (e.g., project number, title and study type).
- Project manager.
- Sampling site name.
- GPS coordinates.
- Type of freshwater water-body (lake, river, reservoir, etc.).
- Name of water-body.
- Sampling date (e.g., DD, MM, YY).
- Sampling time (e.g., HH, MM in a 24-h format).
- Sampling gear type used (e.g. seine netting, gill netting, electro-fishing).
- Sampling depth.
- Scientific name of selected species.
- Common name of selected species.
- Composite sample numbers.
- Number of individuals in each composite sample.
- Number of replicate composite samples.
- Predominant characteristics of specimens used in each composite sample:
    - Predominant life stage of individuals in composite.
    - Predominant sex of individuals in composite (if determined).
    - Mean age of individuals in composite (if determined).
    - Mean body length or size (mm).
    - Description of tissue type (fillets skinned, fillets scaled, whole fish).
- Analytical methods used (including method for lipid analysis).
- Method detection and quantification limits for each selected analyte.
- Sample cleanup procedures (e.g., additional purifying steps for sample extracts or digestives).
- Data qualifiers (e.g., qualifying information about the measurement).
- Percent lipid (wet mass basis) in each composite sample.
- For each selected analyte in each composite sample:
    - Total wet mass of composite sample (g) used in analysis.
    - Measured concentration (wet mass basis) as reported by the laboratory.
    - Units of measurement for selected analyte concentration.
    - Evaluation of laboratory performance (i.e., description of all QA and QC samples associated with the sample(s) and results of all QA and QC analyses).
- In Level 1 surveys (screening surveys) with only one composite sample for each selected species, a comparison between the reported concentration and derived screening concentration (SC) for each selected analyte as well as an indication of whether SC was exceeded must be included.
- In Level 2 surveys (Intensive surveys, Phase I) and Level 3 (Intensive surveys, Phase II) surveys, for each target analyte in each set of replicate composite samples, the following should be included:
    - Range of selected analyte concentrations for each set of replicate composite samples.
    - Mean (arithmetic) selected analyte concentration for each set of replicate composite samples.
    - Standard deviation of mean target selected concentration.

Data Reporting

The project manager should compile the data reports. The report must contain at least the information as compiled in the National Chemical Contaminant Database. However, the project manager must discuss the specific requirements with the people responsible for the risk analyses or with the specific client. If the results are made available to the general public they should be in an easy-to-understand format (Heath, 1999).

STEP 7: RISK ASSESSMENT

Risks are predicted during the risk assessment process by evaluating the inherent ability of a chemical (e.g. chemical contaminant in fish tissue) to cause adverse effects (e.g. health effects in consumers of fish), qualitatively and quantitatively at different concentrations of exposure to the chemical (estimation of the chemical hazard) and the amount of exposure too (estimated exposure) that occurs (Risk *Assistant^{TM}, 1995). Health risk assessments of chemical contaminant levels in freshwater fish thus provide a means of estimating the probability of adverse health effects associated with the measured or estimated level of hazardous contaminants and are a tool for predicting the extent of potential or probable human health effects associated with the eating of the contaminated fish.

The risk assessment process as defined by the National Academy of Sciences of America (NAS, 1983) and recommended by the US EPA (1997) consists of four distinguishable but interacting steps namely, (i) hazard identification, (ii) dose – response assessment, (iii) exposure assessment and (iv) risk characterisation (Figure 9). A description of the above risk assessment steps, in relation to the consumption of contaminated fish, is given by the US EPA (1997). Software packages such as Risk *Assistant^{TM} provide models, databases and other tools required for health risk assessment for chemicals (Risk *Assistant^{TM}, 1995) and can also perform human health risk assessments in relation to the consumption of contaminated freshwater fish.

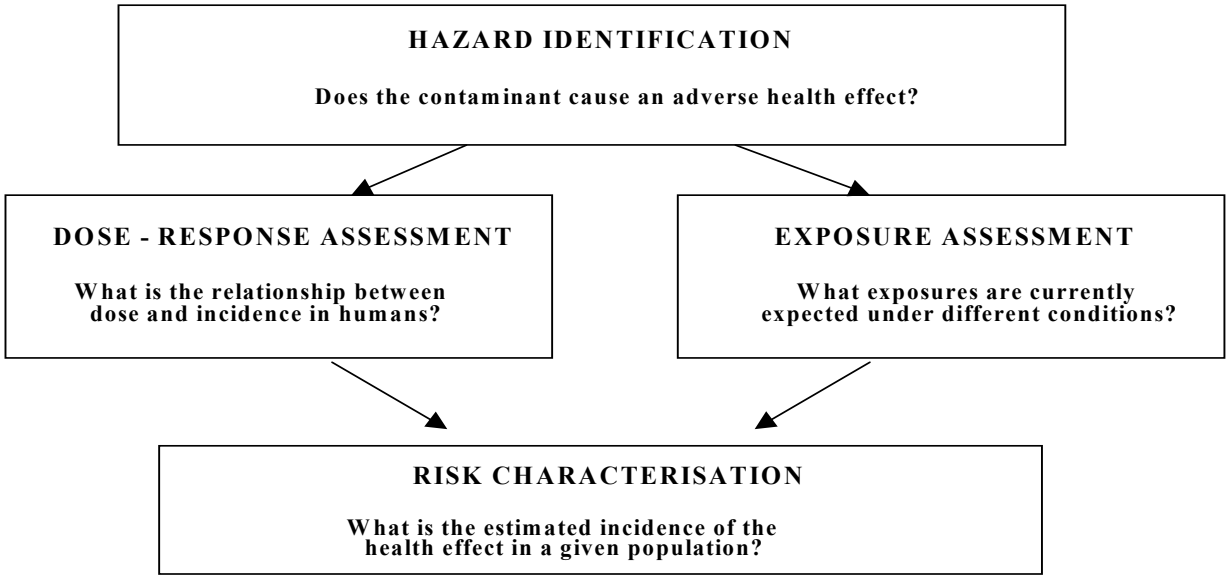


Figure 9. Steps of the risk assessment process (adapted from NAS, 1983)

Hazard identification

This step assesses the likelihood that exposure to a chemical under specific exposure conditions will pose a threat to human health (NAS, 1983; de Beer & Ziolkowski, 1995; US EPA, 1997). General information such as the physical and chemical properties of chemicals, routes and patterns of exposure, structure-activity relationships, metabolic and pharmacokinetic properties, toxicological effects, acute and chronic animal exposure studies, human studies and weight-of-evidence are reviewed in hazard identification (US EPA, 1997). It must be stressed that this information as well as health effects endpoints and related risk values are included in databases such as IRIS and HSDB and should be consulted to obtain this and other information to develop a hazard profile.

The hazard identification steps for chemically contaminated fish would focus on information that is critical in determining the health risk to humans and would review the following:

- Literature and databases (IRIS, 1999; HEAST, 1998; US EPA etc.) on the chemistry and health effects of contaminants. This would include aspects such as oral dose, bioaccumulation potential, persistence and prevalence in the environment, biochemical fate and the human health effects of the chemical contaminant.
- Information on biocide usage and their chemistry and human health effects.
- Data from previous information on contaminant surveys that have resulted in consumption bans or advisories.
- Analytes that have been recommended for fish contaminant monitoring.
- Information obtained from catchment situation analysis of potential and actual point/or diffuse sources of pollution.

It is important to stress that other sources of exposure (for example air, water, soil, workplace, other food including commercially caught fish) may significantly contribute to the overall contaminated exposure. Where such cases are suspected or anticipated a more detailed hazard identification could be undertaken in order to obtain an estimate of total exposure (US EPA, 1997).

During the hazard identification process certain assumptions and simplifications are made which may result in some uncertainty. These include:

- Uncertainty in the estimation of a chemical toxicity if there are insufficient toxicological data to fully characterises the toxicity of the chemical.
- Uncertainty as a result of omitting potential contaminated areas during the surveys (US EPA, 1997).

These uncertainties are usually addressed in the risk assessment steps that are undertaken after the hazard identification step.

To undertake this assessment in South Africa it is recommended that the toxicity databases such as HEALTH EFFECTS SUMMARY TABLES (HEAST, 1998) Agency Toxic Substances and Disease Registry (ATSDR, 1999), Integrated Risk Information System (IRIS, 1999) and Toxicology Excellence for Risk Assessment (TERA, 1999) are used to evaluate the toxicity and carcinogenicity of the various chemical contaminants. The software packages Risk*Assistant™ (Risk*Assistant™, 1995) and the US EPA publication of 1997 (US EPA, 1997) make this information readily available. A review of the following would also provide valuable information:

- Information on biocide usage and its chemistry and human health effects.
- Data from previous information on contaminant surveys that have resulted in consumption bans or advisories.
- Analytes that have been recommended for fish contaminant monitoring.
- Information obtained from catchment situation analysis of potential and actual point/or diffuse sources of pollution.

Dose-response assessment

In this assessment the relationship between the dose of a hazardous chemical (i.e. the amount of the chemical taken into the body through skin contact, breathing and ingestion) and incidence of an adverse health effect in the exposed population is characterised. The dose-response dynamics of a specific chemical and therefore the functional relationship between the exposure and the observed human and/or animal health effects (NAS, 1983; US EPA, 1997) are evaluated.

As stated, hazardous chemicals can be broadly grouped as those with non-threshold effects (causing carcinogenic and genotoxic health effects) and those with threshold effects (causing acute, chronic or developmental effects). A distinction is therefore made in describing the dose-response variables for carcinogenic and non-carcinogenic chemicals.

Carcinogenic effects

It is generally assumed that carcinogens do not have a safe threshold of exposure and any exposure may pose some cancer health risk (US EPA, 1997). Dose-response data obtained from one or more epidemiological studies and/or chronic animal bioassays are used in cancer risk extrapolation models to calculate the cancer slope factor or potency value (SF: the upper 95 percent, upper confidence limit of the slope of the dose-response curve in the low dose-response region). Cancer slope factors (potency factors) have been derived for several chemicals for which sufficient data are available. The slope factors for some of the selected analytes have been derived (US EPA 1995a, 1997). These values can be used to calculate consumption limits. Using oral and inhalation exposure data cancer potencies can also be derived by performing the following steps:

- Identify the most appropriate dose data.
- Modify dose data from interspecies differences.
- Develop an equation describing the dose-response relationships.
- Calculate an upper confidence bound on the data.

These procedures are described in detail in publications such as that of the US EPA (1996a,b, 1997).

Non-carcinogenic effects

Non-carcinogenic effects that occur over a few hours or days are considered to be acute exposure effects, while multiple exposures occurring over a significant period of time are termed chronic exposure effects (IRIS, 1999). Developmental effects are effects on the developing organism and are defined as:

- Death of the developing organism.
- Structural abnormality.
- Altered growth.
- Functional dependency (US EPA, 1997).

Since fish have the ability to bioaccumulate high levels of certain contaminants and recreational and/or subsistence fishermen and their families may consume relatively large and frequent meals of fish, acute exposure is of concern. Information on the minimum levels for effects of some contaminants have been incorporated in the toxicological profiles developed by the ASTDR (ASTDR, 1998, 1999). A summary of acute effects and estimated human lethal doses for most of the selected analytes is given in the publication of the US EPA (1997).

An oral reference dose (RFD) is calculated for the protection against chronic toxicity resulting from exposure to contaminants (US EPA, 1997). RFD is calculated by (i) identifying the most appropriate NOAEL or LOAEL and (ii) applying the relevant uncertainty and modifying factors. More detail on the calculation of RFDs as well as derived RFDs for some of the selected analytes.

To assess developmental toxicity data, toxicity studies on animals are usually used, as data from human studies are not available for most contaminants. To estimate exposure limits for developmental effects the methodology for calculating RFDs can be followed (US EPA, 1997). Additional guidance on estimating exposure limits for contaminants, which cause developmental effects, is provided in the US EPA publication: *Guidelines for Developmental Toxicity Risk Assessment* (US EPA, 1991b). Information on developmental toxicity of some of the selected analytes is provided by the US EPA (US EPA, 1997). However, it must be stressed that the developmental effects of chemicals are less studied than, for example, the carcinogenic effects, thus limiting the development of protective values.

Uncertainties

In developing risk values from dose-response data many assumptions must be made which result in uncertainties. These uncertainties include (but are not limited to):

- Assumptions related to animal-human extrapolations.
- Uncertainty as a result of differences between animal studies and real human populations. Furthermore, human data is usually derived from occupationally exposed people.
- The assumption that there is a threshold for most non-carcinogens and no threshold for carcinogens.
- Uncertainty due to the use of uncertainty and modification factors in calculations.
- Uncertainty due to the assumptions related to the human population (for example, mean body mass, age, daily intake etc.).
- Uncertainty due to the amount and quality of toxicological and epidemiological data available.
- Uncertainty due to the assumptions inherent in the selection of the dose-response model.
- Uncertainty due to the use of the upper-bound estimate of the slope for the carcinogenic slope determinations.

Some of the above-mentioned uncertainties are, however, quantitatively addressed (the application of uncertainty factors, modifying factors or by the use of upper bound estimates) while others can be addressed qualitatively (US EPA, 1997).

Exposure assessment

This assesment is the process of measuring or estimating the intensity, frequency and duration of human exposure to a chemical in potentially exposed populations. A complete exposure assessment deals with (i) the source of the health hazard, (ii) the exposure pathways via various media and routes, (iii) measured or estimated concentrations and exposure duration, (iv) the exposed population and (v) integrated exposure analysis.

Information and data on chemical residues in the fish and human consumption patterns are used to identify and describe potentially exposed populations. The following information and data are therefore used:

- The chemical contaminant (analyte) concentrations in fish that have been determined.
- Geographical distribution of contaminated freshwater fish. This information is required when performing risk characterisation during population exposure assessment and in determining the need for further action.
- Information on where contaminated fish have been found in relation to possible sources of potential contamination (from catchment situation analysis, pollution incidents etc).
- Socio-demographic information (age, sex, body mass, etc.; Table 1and 1.2) and fish consumption patterns (number of species, type of fish, size classes included in the diet, the specific edible portion selected for consumption, fish preparation and cooking methods, meal size and frequency of consuming fish). It is recommended that the general derived values as indicated in Table 2 be used to derive various exposure scenarios (Table 26) for South African water-bodies.

Individual exposure assessment

Individual exposure assessments provide descriptions of the overall media specific or site-specific exposure of the individual (US EPA, 1997). **Exposure** can be estimated from known analyte concentrations in freshwater fish and known human fish consumption patterns as indicated in the following equation:

$$\mathbf{E_m = (C_m \times CR) / BM} \qquad \mathbf{(1)}$$

where:

- E_m = Individual exposure to chemical (analyte) *m* from ingesting freshwater fish (mg/kg/day).
- C_m = Concentration of chemical (analyte) *m* in the edible portion of the species of interest (mg/kg).
- CR = Mean daily consumption rate of the species of interest (kg/day).
- BM =Body mass of an individual consumer (kg).

Table 26. Exposure scenarios that can be developed from the information in Table 2 if the information is not available for a specific population

SCENARIO 1 Mean contaminant concentrations Adult 150 g fish daily	**SCENARIO 2** Mean contaminant concentrations Adult 150 g fish weekly	**SCENARIO 3** Mean contaminant concentrations Adult 50 g fish daily	**SCENARIO 4** Mean contaminant concentrations Adult 50 g fish weekly
SCENARIO 5 Mean contaminant concentrations Child 150 g fish daily	**SCENARIO 6** Mean contaminant concentrations Child 150 g fish weekly	**SCENARIO 7** Mean contaminant concentrations Child 50 g fish daily	**SCENARIO 8** Mean contaminant concentrations Child 50 g fish weekly

Maximum safe human fish consumption rates can be calculated from known chemical (contaminant) concentrations in fish, safety factors (SF) and RFD (dose-response data) values. Therefore, based on a contaminants carcinogenicity the allowable daily consumption rate (one fish species and one type contaminant) of a contaminated fish source is calculated using the following equation (**or can be calculated using EPA documents):**

$$\mathbf{CR_{lim} = (ALR \times BM)/ (SF \times C_m)} \qquad \mathbf{(2)}$$

where:

- CR_{lim} = Maximum allowable fish consumption rate of the species of interest (kg/day). The derived daily consumption limit (CR_{lim}) represents the amount of freshwater fish expected to generate a carcinogenic health risk that is not greater than the maximum acceptable individual lifetime risk level (ALR), assuming that the consumer consumes fish daily at the consumption limit over the persons lifetime.
- ALR = Maximum acceptable individual lifetime risk level (dimensionless).
- BM = Body mass of consumer (kg).
- SF = Oral slope factor or carcinogenicity potency factor $(mg/kg/day)^{-1}$ which is an upper bound risk value.
- C_m = Measured concentration of chemical (analyte) *m* in the edible portion of the species concerned (mg/kg).

To express the daily consumption limits as the allowable number of fish meals (one fish species and one type carcinogenic contaminant) of a given size and over an specified period the following equation can be used:

$$\mathbf{CR_{mm} = (CR_{lim} \times TP_{ap})/ MS} \qquad \mathbf{(3)}$$

where:

- CR_{mm} = maximum allowable fish consumption rate of the species of interest (meals/month).

- CR_{lim} = maximum allowable fish consumption rate of the species of interest (kg/day).
- TP_{ap} = Mean time period (365.25 day/12 months =30.44 day/month).
- MS = meal size (kg fish/meal).

Based on a contaminant's non-carcinogenic health effect the allowable daily consumption rate of (one fish species and one type of non-carcinogenic contaminant) a contaminated fish source is calculated using the following equation:

$$\mathbf{CR_{lim} = (RFD \times BM) / C_m} \qquad \mathbf{(4)}$$

where:

- CR_{lim} = maximum allowable fish consumption rate of the species concerned (kg/day). The derived maximum daily consumption rate (CR_{lim}) represents the amount of freshwater fish which would probably not result in a non-carcinogenic health risk to the consumer over the person's lifetime (US EPA, 1997).
- RFD = Reference dose (mg/kg/day).
- BM =Body mass of consumer (kg).
- C_m = Measured concentration of chemical (analyte) *m* in the edible portion of the species concerned (mg/kg).

Examples of the values of the parameters for the above equations used by the US EPA (1997) to calculate risk-based consumption limits are summarized in Tables 1.5 and 1.6. In these calculations body mass estimates for adult males and non-pregnant females can be averaged. However, for specific contaminants the specific body mass for men, non-pregnant women, women of reproductive age or children should be used. The meal size and consumption rates have been shown to differ between populations, individuals and age groups (adults, children, adolescents).

The frequency of fish consumption is in many cases (for example recreational or subsistence fishermen) not constant over a specified time period as people may consume only contaminated fish for a specific period. To obtain a lifetime average daily dose the cumulative dose over an individual consumer's lifetime is derived by the number of days in an average lifetime. The average period may vary depending on the specific situation, for example an averaging period of 7, 10, or 14 days can be used for recreational fishermen who will only consume the fish during a specific holiday period. However, an averaging period of the month is most commonly used for the expression of meal consumption limits (US EPA, 1997). Local surveys of a specific region may indicate that subsistence and recreational fishermen will consume one contaminated fish specie or several species. To assess the exposure and derive consumption limits for consumers which include more than one species and exposed to one contaminant, the following equation can be used:

$$\mathbf{E_{mj} = \Sigma(C_{mj} \times CR_j \times P_j) / BM} \qquad \mathbf{(5)}$$

where:

- E_{mj} = Individual exposure to chemical (analyte) *m* from ingesting freshwater fish species *j* (mg/kg/day).
- C_{mj} = Concentration of chemical (analyte) *m in* the edible portion of the species *j* of interest (mg/kg).
- CR_j = Mean daily consumption rate of the species *j* concerned (kg/day).
- P_j = Portion of a given fish species in an individual's diet (dimensionless).
- BM =Body mass of an individual consumer (kg).

To derive maximum safe human fish consumption rates for diets that include more than one species for a specific contaminant (more than one species and one contaminant) the doses from each species are added to gathered for all the species eaten proportional to the amount of each species eaten by applying the following equation:

$$\mathbf{CR_{tm} = \sum_{j+1}^{n}(CR_{mj} \times P_j)} \qquad \mathbf{(6)}$$

where:

- CR_{tm} = Total concentration of chemical contaminant *m* in an individual's fish diet (mg/kg).
- CR_{mj} = Measured concentration of chemical contaminant (analyte) *m* in the edible portion of the species *j* concerned (mg/kg).
- P_j = Portion of a given fish species in an individual's diet (dimensionless).

Therefore, based on a contaminant's carcinogenicity the allowable daily consumption of more than one fish species (more than one species and one contaminant) from a contaminated fish source is calculated using the following equation:

$$\mathbf{CR_{lim} = (ALR \times BM) / \sum_{j+1}^{n}(CR_{mj} \times P_j) \times SF} \qquad \mathbf{(7)}$$

where:

- CR_{lim} = Maximum allowable fish consumption rate of the species concerned (kg/day).
- ALR =Maximum acceptable individual lifetime risk level (dimensionless).
- BM =Body mass of consumer (kg).
- CR_{mj} = Measured concentration of chemical contaminant (analyte) *m* in the edible portion of the species of interest (mg/kg).
- P_j = Portion of a given fish species *j* in an individual's diet (dimensionless).
- SF = Oral slope factor or carcinogenicity potency factor $(mg/kg/day)^{-1}$ which is an upper bound risk value.

The maximum safe daily consumption limit of each species (more than one species and one contaminant situation) can be calculated from the following equation:

$$\mathbf{CR_j = CR_{lim} \times P_j} \qquad \mathbf{(8)}$$

where:

- CR_j = Consumption rate of the fish species *j* concerned (kg/day).
- CR_{lim} = Maximum allowable fish consumption rate of the species concerned (kg/day).
- P_j = Portion of a given fish species in an individual's diet (dimensionless).

The meal consumption limits may also be calculated using Equation 3 and substituting CR_j for CR_{lim} in the equation.

Based on a contaminant's non-carcinogenic health effect the allowable daily consumption of more than one fish species (more than one species and one contaminant) from a contaminated site is calculated using the following equation:

$$\mathbf{CR_{lim} = (RFD \times BM) / \sum_{j+1}^{n}(CR_{mj} \times P_j)} \qquad \mathbf{(9)}$$

where:

- CR_{lim} = maximum allowable fish consumption rate of the species concerned (kg/day).
- RFD = Reference dose (mg/kg/day).
- BM =Body mass of consumer (kg).
- CR_{mj} = Measured concentration of chemical contaminant (analyte) *m* in the edible portion of the species of interest (mg/kg).
- P_j = Portion of a given fish species *j* in an individual's diet (dimensionless).

In the aquatic environment the different fish species may be exposed to different contaminants which may be bioaccumulated by them. Consumers of fish from contaminated sites may therefore be exposed to more than one contaminant if they include a specific fish species or more than one fish species in their diet. Based on the contaminant's carcinogenicity the allowable daily consumption of one or more than one fish species contaminated with more than one contaminant (situation: one or more than one species and more than one contaminant) from a contaminated fish source is calculated using the following equation:

$$\mathbf{CR_{lim} = (ALR \times BM) / \sum_{m+1}^{x} [\Sigma (CR_{mj} \times P_j)] \times SF_m} \qquad \mathbf{(10)}$$

where:

- CR_{lim} = Maximum allowable fish consumption rate of the species concerned (kg/day).
- ALR =Maximum acceptable individual lifetime risk level (dimensionless).
- BM =Body mass of consumer (kg).
- CR_{mj} = Measured concentration of chemical contaminant (analyte) *m* in the edible portion of the species *j* of interest (mg/kg).
- P_j = Portion of a given fish species in an individual's diet (dimensionless).
- SF_m = Oral slope factor or carcinogenicity potency factor $(mg/kg/day)^{-1}$ which is an upper bound risk value for chemical contaminant *m*.

If the consumer only includes one species of fish in the diet, Equation 10 may be simplified. Therefore, based on the contaminant's carcinogenicity the allowable daily consumption of one fish species contaminated with more than one contaminant (one species and more than one **contaminant**) from a contaminated fish source is calculated using the following equation:

$$\mathbf{CR_{lim} = (ALR \times BM) / \sum_{m+1}^{x} CR_m \times SF_m} \qquad \mathbf{(11)}$$

where:

- CR_{lim} = Maximum allowable fish consumption rate of the species of interest (kg/day).
- ALR =Maximum acceptable individual lifetime risk level (dimensionless).
- BM =Body mass of consumer (kg).
- CR_m = Measured concentration of chemical contaminant (analyte) *m* in the edible portion of the species of interest (mg/kg).
- SF_m = Oral slope factor or carcinogenicity potency factor $(mg/kg/day)^{-1}$ which is an upper bound risk value for chemical contaminant *m*.

The meal consumption limits based on the contaminant's carcinogenicity may also be calculated using Equation.3.

If contaminants have similar non-carcinogenic health effects, the allowable daily consumption of one or more than one fish species contaminated with more than one contaminant (situation: one or more than one species and more than one contaminant) from a contaminated fish source is calculated using the following equation:

$$\mathbf{CR_{lim} = \sum_{m+1}^{x} [RFD_m / \sum_{j+1}^{n} (CR_{mj} \times P_j)] \times BM} \qquad \mathbf{(12)}$$

where:

- CR_{lim} = Maximum allowable fish consumption rate of the species concerned (kg/day).
- RFD_m = Reference dose of contaminant *m* (mg/kg/day).
- BM =Body mass of consumer (kg).
- CR_{mj} = Measured concentration of chemical contaminant (analyte) *m* in the edible portion of the species *j* concerned (mg/kg).
- P_j = Portion of a given fish species in an individual's diet (dimensionless).

If the consumer only includes one species of fish in his or her diet, Equation 12 may be simplified. Therefore, for contaminants that have a similar non-carcinogenic health effect, the allowable daily consumption of one fish species contaminated with more than one contaminant (one species and more than one contaminant) from a contaminated fish source is calculated using the following equation:

$$\mathbf{CR_{lim} = \sum_{m+1}^{x} [RFD_m / (CR_m)] \times BM} \qquad \mathbf{(13)}$$

where:

- CR_{lim} = Maximum allowable fish consumption rate of the species concerned (kg/day).
- RFD_m = Reference dose of contaminant *m* (mg/kg/day)
- BM = Body mass of consumer (kg).
- CR_m = Measured concentration of chemical contaminant (analyte) *m* in the edible portion of the species concerned (mg/kg).

Risk based consumption limits can therefore be derived for contaminants using equation 1 to 13, risk values and selected input values (Table 6). Examples of the tables that can be compiled are presented in Table 27 and Table 28. This approach was used by the US EPA (1997) to derived risk-based consumption limits for each of the selected analytes (Table 5). These risk based consumption limits can be used as a guide to the consumption of South African freshwater fish for the selected analytes if information on the input values and risk values is not available for South African situations.

Population exposure assessment

Population exposure assessments are usually used in risk management and to identify sections of the population of interest and not for developing risk-based consumption limits (US EPA, 1997). To evaluate population exposures the following information is required:

- Basic socio-demographic data. The age, sex, body mass distribution of the population where exposure is likely to occur.
- Food preparation and cooking methods. Exposure intake of some chemical contaminants can be reduced by skinning and trimming the fish fillets or by cooking the fish.
- Information on multimedia exposure. In some areas it is therefore important to obtain information on the overall exposure to sources other than fish chemical contaminants. These sources include (but are not limited to) occupational, airborne, soil, drinking water or other non-fish foods. The following equation may be used to express total exposure:

$$E_T = [(C_m \times CR) / BM] \times E_A \times E_W \times E_F \times E_O \qquad (14)$$

where:

- E_T = Exposure to all sources (mg/kg/day) to contaminant *m*.
- C_m = Concentration in the edible portion of the species concerned (mg/kg).
- CR = Mean daily consumption rate of the fish species concerned (kg/day).
- BM = Body mass of an individual consumer (kg).
- E_A = Exposure from air sources (mg/kg/day).
- E_W = Exposure from water sources (mg/kg/day).
- E_F = Exposure from nonfish food sources (mg/kg/day).
- E_O = Exposure from other sources (mg/kg/day). = Exposure from air sources (mg/kg/day)

Table 27. An example of a summary table of the monthly consumption limits for carcinogenic health endpoints for the general population (adapted from the US EPA, 1997)

CHEMICAL CONCENTRATION IN FISH TISSUE[a] (mg/kg)	RECOMMENDED RISK – BASED CONSUMPTION LIMIT (MEALS PER MONTH)								
	Meal size 0.050 kg			Meal size 0.150 kg			Meal size 0.500 kg		
	ARL			ARL			ARL		
	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}

ARL = Acceptable risk level.
NONE = No consumption recommended.

INFORMATION IN TABLE PRESENTED AS:
6/yr = Consumption of no more than 6 meals per year is recommended.
>30 = Consumption of more than 30 meals/month is allowed, it is advisable to limit consumption to 30 meals in 1 month (1 meal per day).
[a] Shaded values are below detection limit, _____ mg/kg.
All values were rounded down to the nearest whole meal size.
Consumption limits are calculated based on an adult body mass of _____ kg and using a cancer potency factor of _____ per mg/kg/day.

Table 28. An example of a summary table of the monthly consumption limits for systemic health endpoints for adult males in the general population (adapted from the US EPA, 1997)

CHEMICAL CONCENTRATION IN FISH TISSUE (mg/kg)	RECOMMENDED RISK – BASED CONSUMPTION LIMIT (MEALS PER MONTH) [a]			
	Meal size 0.100 kg	Meal size 0.150 kg	Meal size 0.250 kg	Meal size 0.500 kg

NONE = No consumption recommended.
INFORMATION IN TABLE PRESENTED AS:

6/yr = Consumption of no more than 6 meals per year is recommended.
>30 = Consumption of more than 30 meals/month is allowed, it is advisable to limit consumption to 30 meals in 1 month (1 meal per day).
Consumption limits are calculated based on an adult body mass of _____kg and using RFD = _____ mg/kg/day.
Shaded values are below the detection limit, _____ mg/kg.
All values were rounded down to the nearest whole meal size.
[a] Monthly limits are based on the total dose allowable over a 1-month period (based on the RFD). When this dose is consumed in less than 1 month (e.g., in a few large meals), the daily dose will exceed the RFD.

- Fish consumption and consumption patterns. Local information on fish consumption patterns throughout the population as well as the concentration of analytes in the fish tissue of a fish specie (size class) and from an identified water-body.
- General nutritional status of the different segments of the population under investigation. This information is obtained because people with a poor nutritional status are usually at greater heath risk from many chemicals.

More detail on the methodology for the calculations of total exposure can be found in the US EPA publications (US EPA, 1991b; 1997). Population exposure assessments usually include individual consumers at the central tendency and high-end portion of the exposure distribution (Figure 10). The central tendency represents the average exposure in the specific population and is derived from either the arithmetic mean or the median exposure level. When exposure distributions are skewed, as many are, the median value (e.g. the geometric mean) is a better indicator of the midpoint of the exposure distribution. It must be stressed that the central tendency is less appropriate for evaluating non-cancer health risks (based on threshold exposures) as consumers exposed at levels above the average level may have exposures higher than the threshold for health effects (US EPA, 1997).

The high-end estimates of exposures are estimates of individual exposures at the upper end of the exposure distribution and lie between the 90th and 99.9th percentiles (Figure 10). These estimates are important in describing population risks and establishing exposure limits as they represent reasonable worst-case scenarios (US EPA, 1997). The bounding estimates - that is estimates greater than the highest actual estimate (≥ 99.9th percentile of the exposed population) are important in evaluating the upper bound risk but are not recommended for use in estimating risks associated with consumption of contaminated fish by the US EPA (1997).

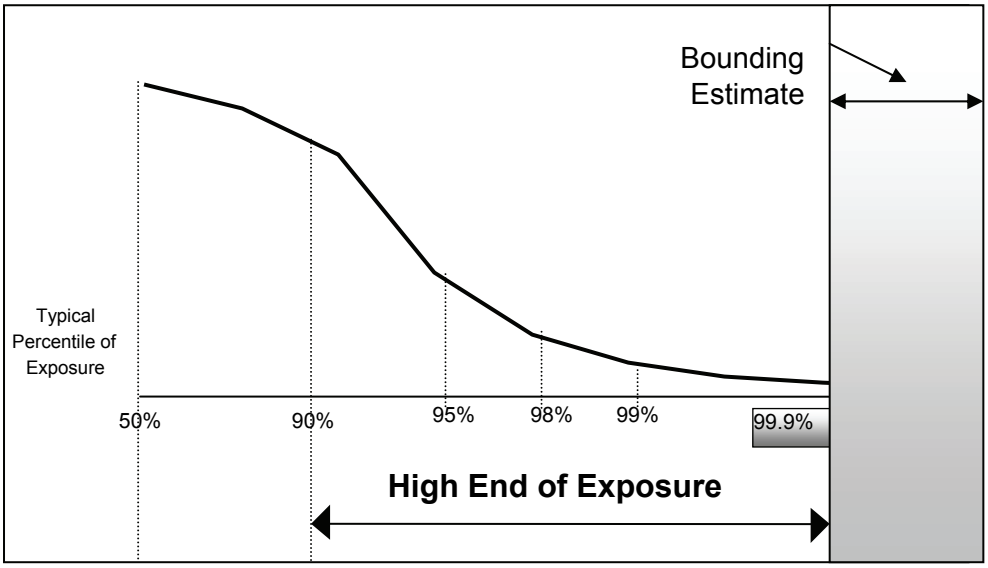


Figure 10. Schematic presentation of exposure categories in the upper half of a normal distribution (adapted from the US EPA, 1997)

Uncertainty

During exposure assessment several assumptions are made which result in uncertainty. These uncertainties include, but are not limited to:

- Variation in chemical contaminant concentrations in fish from a specific water-body.
- Uncertainty as a result of the accurate measurement of analytes.
- Uncertainty in demographic information of the consumer population (body mass, age, sex) specific fish population.
- Uncertainty in fish consumption statistics, for example data on the size and frequency of meals, the species consumed, which portion of the fish is included in the diet and the methods of preparation and cooking of the fish.
- Uncertainty in exposure assessment when consumers are exposed to other contaminant sources (from water, from air, food other than fish, soil, etc.).
- Uncertainty if exposure limits consider only one fish species when the consumer population includes more than one species in their diet.

The exposure assumptions and the uncertainties must be evaluated to ensure that the uncertainty factors and assumptions adequately protect the sensitivities of highly exposed consumer populations. However, it must be stressed that for South African conditions much of the information and data will not be adequate to perform the proposed calculations. It is therefore recommended that for South African programmes the proposed information requirements are evaluated and obtained where feasible, considering the specific programme objectives and availability of resources.

Risk characterisation

In this assesment in the risk assessment process all the information about the other three steps (hazard identification, dose-response assessment, exposure assessment) is used to characterize and describe the extent of the overall individual or population risk. The quantitative and qualitative aspects of the risk assessment, the assumptions used and the identification of uncertainties are thus assessed and discussed to provide an overall estimate of individual and population risk (Browner, 1995; US EPA, 1995b, 1997). To achieve the above-mentioned and to give some guidance to perform a general risks characterisation step for chemicals, several issues should be addressed. Some of the most important issues are discussed in the sections that follow.

Characterisation of hazard identification

- The key toxicological studies that provide the basis for health concerns are identified and the following are addressed:
    - Define the origin of the data by indicating if the data are from field or laboratory studies and if single or multiple species are involved.
    - Explain the scientific merit of these studies.
    - For a carcinogenic hazard comment on the observation of single or multiple tumor sites, occurrence of benign or malignant or non-carcinogenic tumors, the use of maximum tolerated dose, etc.
    - For non-carcinogenic hazards identify the endpoints and the basis for the selection of critical effects.

  - Discuss any valid studies that support or conflict with the study.
- Consider if there is any other health endpoint of concern and indicate if there are any significant data gaps.
- Discuss available epidemiological and clinical data and focus on the following:
  - Types of studies that were used, for example ecological, case-control, cohort, etc.
  - Degree to which exposures were described.
  - Degree to which confounding factors were accounted for.
  - Degree to which other causal factors were excluded.
- Discuss how much is known about how (biochemical and/or biological mechanisms) the chemical produces adverse effects. The following issues should be addressed:
  - Information on mechanisms of action or metabolism.
  - The implications for possible health effects.
  - How the information aids in the interpretation of the toxicity data.
- Discuss the non-positive data in animals and humans and whether these data were used in the hazard identification.
- Characterise the observed health effects in wildlife species and discuss the important issues as indicated in the points above.
- Finally, summarise the hazard identification and discuss the relevance of the following:
  - Confidence in the conclusions.
  - Alternative conclusions supported by the data.
  - Data gaps.
  - Major assumptions.

Characterisation of dose-response assessment.

- Provide information related to the data used to derive the dose-response curve and assess whether the result would have been significantly different had it been based on different data sets. The following should also receive attention in this assessment:
  - If animal data were used, name the species and indicate if the most sensitive species or average of all species or other were used. Provide reasons for selection.
  - If epidemiological data were used state which studies were used and indicate if only positive studies or all studies or some other combination were used. Give details on studies that were excluded and why. Discuss the procedures of meta-analysis if it was performed.
- Provide information on the model that was used to develop the dose-response curve and explain on what basis it was selected. Explain the chemical-specific information available to support the approach.
  - For non-carcinogenic hazards state the calculation of RFD, the assumptions or uncertainty factors used and the confidence in the estimates.
  - For carcinogenic hazards state the dose-response model used, the selection criteria for the specific model and if other dose-response models were considered.
- Discuss the route and level of exposure observed, as compared to the expected human exposure.
  - If data are not available for the same route as for the expected human exposure, indicate whether pharmacokinetic data are available to extrapolate

across route of exposure. Discuss the consequences of such an extrapolation.

- Characterise wildlife dose-response information if health effects were observed in wildlife species and discuss the important issues as indicated in the points above.

Characterisation of exposure assessment

- The most significant aspects of environmental exposure are identified. The possibility of exposure to fish contaminants from other sources, for example food other than fish, water, soil, air, occupational activities, etc is thus evaluated. The following are therefore addressed:
  - Discuss the data available from the different sources of exposure from the various media and explain the contribution of each exposure.
  - Indicate the most significant environmental pathways for exposure.
- Describe the general population, highly exposed sub-populations and highly susceptible groups that were assessed.
- Describe the basis for the exposure assessment including any monitoring, modelling or other analysis of exposure distributions.
- Discuss the key descriptors of the exposure. The following should be addressed:
  - Describe the exposures to average individuals, high-end individuals, high exposure groups, children, susceptible populations, etc. Explain how the central tendency and high-end estimate were derived.
  - Discuss all relevant information related to the populations assessed.
  - A description and a discussion of the health risks to individuals and populations in terms of the severity and extent of possible harm. Special attention should be given to the characteristics (age, sex, nutritional status, general health status, etc) of sub-populations that make them more susceptible than the general population.
- Discuss the concerns regarding cumulative or multiple exposures in relation to ethnic, racial or socio-economic considerations. Evaluation of information from local medical practitioners to identify possible health related risks could also provide useful information.
- Characterise wildlife exposure information if health effects were observed in wildlife species and discuss the important issues as indicated in the points above.
- Summarise exposure conclusion and discuss the following:
  - The results of the different approaches, the limitations of each, the range of most reasonable values, the confidence in the results obtained and the limitations to the results.

Risk conclusions

- Derive and describe the possible cancer risks
  - If the chemical is ingested. For example, by the consumption of contaminated freshwater fish, risk is calculated as a function of the chemical's oral slope factor (SF). Individual lifetime cancer risk can also be described as:

Individual lifetime cancer risk = Exposure x Cancer slope factor or cancer potency

where:

- Exposure = Total exposure to a single chemical contaminant from all sources (mg/kg/day).
- Cancer slope factor or cancer potency = Upper bound of the lifetime cancer risk (mg/kg/ day).

The population cancer risk can be calculated as:

Population cancer risk = Individual lifetime cancer risk x Size of the exposed population

When different exposure levels occur the total risk is the sum of the risk at each level:

Total population cancer risk = Risk at exposure level *a* + Risk at exposure level *b* + … Risk at exposure level *n*

When multiple contaminants exposures occur, the total risk is equal to the sum of the risks from individual contaminants at each level.

Total population cancer risk = (Risk of contaminant *a* at exposure level *a* + Risk of contaminant *b* at exposure level *a* + … Risk of contaminant *m* at exposure level *a*) + … (Risk of contaminant *a* at exposure level *n* + Risk of contaminant *b* at exposure level *n* + … Risk of contaminant *m* at exposure level *n*).

These cancer risks are frequently expressed as *unit cancer risk* (for individuals or populations), representing the excess lifetime risk due to constant lifetime exposure of one concentration unit of the carcinogen. The unit cancer risk is calculated by the following equation:

$$\text{Lifetime cancer risk} = 1 - e^{-(\text{Exposure} \times \text{Cancer slope factor or cancer potency})}$$

- Derive and describe the possible non-cancer risks
  - For chemical contaminants that cause non-carcinogenic effects a *hazard quotient* (HQ) is calculated. The HQ compares the expected exposure to the chemical contaminant to an exposure that is assumed not to be associated with a toxic effect. If the chemical is ingested for example by the consumption of contaminated freshwater fish (oral exposure), the average daily dose (ADD) is compared to the reference dose (RFD). Individual lifetime cancer risk can also be described as:

$$\text{HQ} = \text{ADD}/\text{RFD}$$

where:

- HQ = Hazard quotient for individual lifetime cancer risk.
- ADD = The average daily dose.
- RFD = The reference dose.

or presented as:

HQ = Exposure/RFD

where:

- HQ = Hazard quotient for individual lifetime cancer risk.
- Exposure = Total exposure to a single chemical contaminant from all sources (mg/kg/day).
- RFD = Reference dose or any other non-carcinogenic exposure limit.

When exposure exceeds the RFD, that is the HQ is equal to or greater than 1.0 (for a single chemical contaminant or for a combination of chemical), the possibility of non-cancer risks from the exposure is indicated. In most cases the less serious effects will become more serious as exposure exceeds the RFD.

Population non-cancer risk can be defined by the following equation:

Non-carcinogenic risk = Population with exposure greater than the RFD

It is important to note that total exposure information can give a more accurate assessment of risk. Risk can also be described by comparing the NOAEL to the estimated dose. The dose is thus expressed as the magnitude by which the NOAEL exceeds the estimate dose (termed the margin of exposure). If the margin of exposure (MOE) is greater than the product of the uncertainty and modifying factors used to calculate the RFD from the NOEL, then the risk is low.

- Based on the hazard identification, dose-response and exposure characterisations describe the overall picture of risk.
- Based on the hazard identification, dose-response and exposure characterisations describe the major conclusions, the major limitations, uncertainties and strength of the assessment.
- The evaluation of the overall quality of the assessment and the degree of confidence in the estimates of health risks and the conclusions made. The uncertainties, limitations and assumptions related to the process are therefore discussed. The possible unavailability of data for health endpoints for aspects such as developmental abnormalities, neurotoxicity and immunotoxicity.
- Indicate if alternative approaches were evaluated and explain the reasons for the specific selections.

Risk context

- Discuss the qualitative characteristics of the hazard and explain the alternatives to these hazards.
- Compare this risk to other risks.
- Discuss the important community concerns, which influence public perception of the risk.
- In fish consumption advisories a discussion on the possible nutritional and socio-economic impacts that may occur if the consumption of fish is restricted or banned. However, this should be addressed in detail when addressing risk management options.

Existing risk information.

- Make reference to any other similar risk assessments and comment on any similar or different conclusions.

Communication of results and other relevant information to risk managers.

- Supply other information that would be useful to the risk manager or the general public (Browner, 1995; US EPA, 1997,1995b).

From the preceding it is evident that the risk characterisation step of the risk assessment process for freshwater fish contaminants entails the integration of the information from the individual characterisation of the other three steps (hazard identification, dose-response assessment, exposure assessment) of the risk assessment process by using quantitative information, qualitative information and information related to uncertainties.

Documenting and summarising risk data

During the risk assessment process much data and results will be generated that should be documented and organised in a way that will facilitate their review and assessment. Although specific projects will require different forms depending on the specific risk assessment undertaken, the following documents should give some guidance (US EPA, 1997):

- Exposure data summary table (Table 29)
- Risk estimates summary table (Table 30)
- Risk characterisation summary table (Table 31)
- Risk summary table for a specific water-body (Table 32)
- Risk summary table for a catchment or geographical area (Table 33)

It is therefore recommended that the risk assessment project leader or designated person design specific forms to ensure proper documentation.

*The application of the Risk*Assistant^{tm} software package in risk assessment*

The Risk*Assistant^{TM} is a computer software package that enables the risk assessor to assess the health risk as a result of exposure to chemicals from different environmental media and some food groups (surface water, groundwater, sediment, soil. air, fish, meat, dairy products, vegetables and fruit). Both cancer health risks (that is, the likelihood of a person getting cancer due to the exposure to a specific chemical) and non-cancer health risks (that is, due to exposure to a chemical which cause non-carcinogenic health (toxic) effects) can be derived by using tools and databases supported by Risk*Assistant^{TM} (Risk*Assistant^{TM}, 1995). To derive these health estimates and exposures, the software package (i) applies standard approaches to generate estimates of exposure, (ii) uses available data on the predicted proportioned relationship for cancer effects at low doses and/or a refined dose below which non-cancer adverse effects are not shown for a specific chemical, (iii) uses relevant local information and (iv) tests many alternative incorporated assumptions.

Table 29. An example of an exposure data summary table (adapted from the US EPA, 1997)

Location:

Date of exposure summary:

Population subgroup (e.g., children, women 18 – 45 year, etc.):

Population size:

Body mass:

Conta-minant (level)	Fish exposure estimates (mg/kg/day)		Other exposures								Subtotal of other exposures (mg/kg/day)		Total of all exposures (mg/kg/day)	
			Air (mg/kg/day)		Water (mg/kg/day)		Food (mg/kg/day)		Other (e.g., soil) (mg/kg/day)					
	Central	High end [a]	Central	High end	Central	High end	Central	High end	Central	High end	Central	High end	Central	High end

[a] Risk assessors may wish to use a bounding estimate rather than a high end estimate (or both)

Table 30. An example of a risk characterisation summary table (adapted from the US EPA, 1997)

Location:_____

Date of information summary: _____

Population: _____

Population Size: _____

Contaminant level (mg/kg)	**TOTAL**					
	Central tendency			**High-end estimate or bounding estimate**		
	Carcinogen (Lifetime risk)	Noncarcinogen (% of RFD)	Alternatives (% of alternatives)	Carcinogen (Lifetime risk)	Non-carcinogen (% of RFD)	Alternatives (% of alternatives)

Table 31. An example of a risk estimates summary table (adapted from the US EPA, 1997)

LOCATION:
DATE:
POPULATION:
POPULATION SIZE:
CONTAMINANT:
CONTAMINANT CONCENTRATION:

Specific subgroup	Fish exposure estimates		Other exposures	Subtotal of other exposures		Total all exposures		Risk values			Other factors (e.g., special susceptibili-ties due to nutritional status, disease, etc.)
	Central tendency	High-end [a]		Central tendency	High-end	Central tendency	High-end	Non-carcinogen	Carcinogen	Alter-natives	
Total population											
<18 year											
>18 year											
Women 18-45 years											

Risk estimate

Central tendency						High-end estimate					
Non-carcinogen (% of RFD)		Carcinogen (Lifetime risk)		Alternatives (% of alternatives)		Non-carcinogen (% of RFD)		Carcinogen (Lifetime risk)		Alternatives (% of alternatives)	
Fish Only	All Exposures	Fish Only	All Exposures	Fish Only	All exposures	Fish only	All exposures	Fish only	All exposures	Fish only	All exposures

[a] Bounding estimate may also be used.

Table 32. An example of a risk summary table for a specific water-body (adapted from the US EPA, 1997)

DATE:	**RISK ESTIMATES BASED ON HIGH-END EXPOSURES**		
POPULATION GROUP	**CANCER RISKS**	**NON-CANCER RISKS**	**OTHER RISKS**
Total Population A <18 year >18 year Women 18-45 year			
Total Population B <18 year >18 year Women 18-45 year			
Total Population C <18 year >18 year Women 18-45 year			
Aggregate of A,B,C <18 year >18 year Women 18-45 year			

Table 33. An example of a risk summary table for a geographic area (adapted from the US EPA, 1997)

DATE:	RISK ESTIMATES BASED ON HIGH-END EXPOSURES	
WATER-BODY LOCATION	**CARCINOGENIC EFFECTS**	**NON-CARCINOGENIC EFFECTS**
TOTAL RISK:		

The Risk*Assistant™ software package therefore:

Supplies information and data on chemical concentrations in one or more environmental media and selects a method to calculate representative data for the local situation. Risk*Assistant™ can therefore:

- Calculate the environmental concentrations to be used in the exposure assessment or use the supplied information (e.g. for a specific local condition) on environmental releases and/or chemical contaminant concentrations for use in the calculations.
- Supports the IRIS, HEAST, New Jersey toxic hazard database and the California Environmental PA (Proposition 65) toxic hazard database. The risk assessor can therefore select a specific hazard database for use in the risk assessment or can enter his/her own values.
- Estimates exposure for the risk assessment. The risk assessor can indicate various applicable scenarios and supply information on local conditions and assumptions.
- Provides a means by which to select from many options, tables, graphs and text components the information the assessor would like to include in a report. This information is available on-screen, stored on disc or printed (Risk*Assistant™, 1995).

This software package therefore provides assistance in performing human health risk assessments. The Risk*Assistant™ software package is also flexible and incorporates data on chemical concentration in the local environment and enables the risk assessor to consider a

wide range of possible exposure causes. Furthermore, the risk assessor can immediately perform sensitivity analysis to test the impact of different assumptions on exposure health risks. In South Africa, Claassen (1996), Heath (1999) and du Preez (2000) used the Risk*Assistant™ software package to assess the possible health risk associated with the consumption of fish from the Crocodile, Olifants, Levuvhu, Sabie, Berg,Vaal rivers. These studies showed that this programme can be effectively applied to South African conditions and should be applied to fish contaminant data. Currently the CSIR (Division of Water Environment and Forestry: Stellenbosch) has competent personnel and the infrastructure to apply the Risk*Assistant™ software package to fish chemical contaminant data.

To perform these risk calculations for the chemical contaminants found in freshwater fish from South African systems and for different scenarios **it is recommended** that the Risk*Assistant™ software package (with assistance from the competent personnel at the CSIR) is used.

STEP 8: RISK MANAGEMENT

In the context of the consumption of chemically contaminated freshwater fish, risk management aims to minimise the health risk to fish consumers (especially highly exposed individuals or population groups) as well as the negative effects that restricting consumption may have (US EPA 1996d). However, the long-term goal must be to reduce the impacts on the water-body to such a level that the contaminant levels in the fish pose no health risk to consumers.

Evaluation of risk assessment data and information

The risk manager evaluates and familiarises himself with the data and information obtained during the risk assessment process. Special attention is given to the assumptions and uncertainties identified during the risk assessment process. Furthermore, it is also essential that the risk manager familiarise himself with the sample collection and analysis programme.

Assessment of the risk management options

The risk manager can select from a variety of options to limit consumption of contaminated freshwater fish, thereby reducing the health risk to consumers. Since no single approach is appropriate for all circumstances it is recommended that the following options are considered for South African water-bodies:

- **No action**. Unlimited fishing is allowed under this option. This option should only be considered when the risk assessment indicates no action is required.
- **Fish consumption advisory**. Information is supplied to the consumers that will lead to the voluntarily restrict their consumption of fish to safe levels. Two types of fish consumption advisories (namely, 'general' and 'quantitative') can be used. General fish consumption advisories provide qualitative guidance on reducing risk through selective fishing, cooking and preparation techniques. In addition to this information quantitative advisories provide consumers with specific information (related to site, species and size) regarding the maximum amount of fish that can safely be consumed over a period.

- **Catch and release**. This option is followed if the consumption of contaminated fish by recreational fishermen is a major concern. Fishing is thus allowed but the anglers are encouraged or forced to release fish after capture. The recreational aspect of fishing is therefore less impacted upon.
- **Fishing ban**. This option is usually followed when the contaminant levels pose a very high health risk. It involves the banning of fish by closing water-bodies to fishing and/or banning the possession of contaminated fish (US EPA 1996b).

The feasibility, efficacy and resource cost of these risk management options differ substantially and must therefore be evaluated. Table 34 gives some guidance as to the feasibility and efficacy of these risk management options.

Assessment of the positive and negative impacts of the risk management options

The risk manager must assess the numerous impacts of the risk management options to limiting the consumption of freshwater fish. In many cases the impacts are site-specific and will depend on local conditions, for example, the population, the economy, and social and cultural factors, to mention only a few (US EPA, 1996). Since no single approach is appropriate for all circumstances it **is recommended** that the following possible impacts of the risks management options must be considered by the risk manager for South African conditions:

- **The impact on the basic nutritional needs of the target population and associated health benefits from eating fish**. Fish are generally beneficial as they provide an excellent source of protein and vitamins. Other health benefits include a decrease in cardiovascular decease, reduced risk of colon cancer and breast cancer, and reduction in high blood pressure to mention only a few. In this evaluation the risk manager must consider the present health status of the target population, and their capacity to substitute fish with other food sources (availability of alternative food sources, economic capacity, etc.).
- **Cultural and social impacts**. Fishing and fish consumption may be part of the traditional activities of the affected population (for example, the indigenous people of Maputaland and KwaZulu Natal Provinces). Fishing may also be a major part of their economic and nutritional base. Furthermore, in South Africa recreational fishing is a primary hobby for many people in which the whole family participates.
- **Economic impact: cost of fishing**. The potential financial losses due to recreational fishing industry. General increase in cost for recreational fishermen that must visit other uncontaminated water-bodies.
- **Economic impact: cost of food**. Subsistence fishermen may also experience hardship, as alternative protein sources will be more expensive. The local community as a whole may suffer if fish is their main protein source.
- **Economic impact: cost on tourism**. Local tourism will decline as recreational fishermen may be forced to visit other uncontaminated water-bodies.
- **Economic impact: cost associated with property values**. The property value of land adjacent to the water-body that is affected by the limitation on fishing may be negatively affected.

From the preceding it is evident that the risk manager must carefully evaluate all the benefits and negative impacts of the various risk management options. The risk manager must discuss the various options with policy-makers, community leaders and community members

(interested and affected parties) to ensure they have a good understanding of the possible impacts that may occur as a result of the various options put forward.

Table 34. Summary of the feasibility and efficacy of the proposed risk management options (adapted from the US EPA, 1996b)

RISK MANAGEMENT OPTIONS		FEASIBILITY			EFFICACY	
		Staffing	**Funding**	**Regulatory authority required**	**Consumer education**	**Source specific risk reduction**
No action required		N/A	N/A	No	None	None
Fish consumption advisory	General guidance	Moderate	Moderate	No	Moderate	Low to moderate
	Quantitative guidance	Moderate to high	Moderate to high	No	Moderate to high	Moderate to high
Catch and release	Voluntary	Low to high	Low to high	No	Low to high	Low to high
	Mandatory	High	High	Yes	Low to high	High
Fishing ban	Voluntary	Moderate to high	Low to high	No	Low to high	Low to high
	Mandatory	High	High	Yes	Low to high	High

Selection of the most appropriate action and recommendations

The final selection of the most appropriate management option is the most critical decision the risk manager has to make. The selection is based on all the information collected and assessed during all the previous phases of the project. It is important that all the resource implications and the practicality of all the risk management options form part of this final analysis.

After consensus between the interested and affected parties (policy-makers, scientific and health advisors, community leaders and members of the community) and the risk manager has been reached the most appropriate risk management option for dealing with the consumption of freshwater fish is made by the risk manager. Recommendations regarding the remedial action to be taken in order to reduce the chemical contaminant load in the water-body and ultimately in the fish population can also be made. This information would be of great value in focusing some of the catchment management objectives of other programs, for example the National River Health Programme and that of the Catchment Management Agencies as stipulated in the Water Act (36/ 1998).

It is recommended that in South Africa the final risk management option and the additional recommendations be submitted to the Department of Water Affairs and Forestry for implementation. The implementation by the Department of Water Affairs and Forestry will however be done in collaboration with other governmental organisations (the Department of Health, provincial environmental, nature conservation and tourism departments, local governmental structures) and the Catchment Management Agencies.

STEP 9: RISK COMMUNICATION

In the context of the consumption of chemically contaminated freshwater fish risk communications aims to 'share' information between all role-players to minimise the health risk to fish consumers (US EPA, 1995b). The development and implementation of a risk communication strategy usually involves: (i) problem analysis during which the risk communicator familiarises himself with the programme; (ii) audience needs assessment (identification of the target population, their specific information needs and the best way to communicate with them); (iii) communication strategy design and implementation (addresses the what, how, when and by whom of communication); and (v) continuous evaluation of the programme (US EPA, 1995b). Presently it would not be feasible to develop a complete risk communicate strategy and implement this for South African water-bodies as it is time-consuming and resource-intensive.

The following is therefore recommended for risk communication related to freshwater fish for South African surveys:

- **The risk communicator uses** the risk assessment and risk management documents and personal judgement to (i) familiarise himself with the programme, (ii) identify the target populations needs.
- **The risk communicator selects** the main consumption information by considering the following:
    - Frequency of consumption of fish from a specific water-body or water-bodies.
    - The human health benefits from eating fish.
    - Chemicals of specific concern and their human health effects.
    - The adverse health effects of eating contaminated freshwater fish.
    - Reducing fish consumption risk by cleaning and cooking methods.
    - Identification of the safer fish species or size of fish or the water-bodies that have the lowest contamination.
- **The risk communicator selects** the style of presenting the information by considering the following:
    - A combination of text tables and diagrams or graphics is the most effective.
    - Using a **persuasive** tone and not a commanding tone.
    - Assessing whether qualitative or quantitative information is the most suitable for the target group.
    - Determining what the education level of the target group is, for example, will they be able to read the information.
- **The risk communicator selects** the most appropriate dissemination mechanism by considering the following:
    - Mass media types: for example, talks on the local radio stations and on television (for example 50/50 programme on SABC).
    - Specialised media types for example brochures, posters, fact sheets, newsletters, fishing regulation articles in newspapers and fishing and outdoor magazines.
    - Interpersonal contacts during meetings of non-governmental organisations, town councils, fishing clubs and catchment forum meetings or during contacts with the staff of the Department of Water Affairs and Forestry and other governmental organisations (the Department of Health, provincial

environmental, nature conservation and tourism departments) responsible for information transfer in the area of interest.

- **The risk communicator** selects the most appropriate time for information change by considering the following:
    - Information exchange should be done throughout the year to stimulate public awareness and keep up compliance.
    - Target specific times of the year, for example the beginning of spring when fishing generally starts and the summer period when fishing is most intensive.
    - Summer holidays or days when fishing competitions are held.

As resources becomes more available the risk communicator should expand the specific programme for example by obtaining detailed information needs from the target group or by producing additional and more detailed information. **It is recommended** that in South Africa the risk communication related to the consumption of contaminated freshwater fish is managed and implemented by the Department of Water Affairs and Forestry in collaboration with other governmental organisations (the Department of Health, provincial environmental, nature conservation and tourism departments, local governmental structures) and the Catchment Management Agencies.

STEP 10: EVALUATION AND REVIEW OF PROGRAMME

It is vital to formally review all aspects of the programme. Specific attention must be given to the following:

- **Re-assessment of the fish contaminant data** obtained during the follow-up surveys.
- **Re-assessment of the risk assessment process** as new information may be available and as new data is obtained.
- **Re-assessment of the risk management programme** to determine whether the programme was and still is effective.
- **Re-assessment of the risk communication initiatives** to determine whether the objectives of the communication strategy were reached.

The review must also consider the objectives, activities and remedial actions that have been taken by other programmes, especially those related to catchment management and the River Health programmes of the Department of water Affairs and Forestry. This review will enable the risk manager to adapt the programme as required thereby achieving the goals of reducing the health risk to the consumers of freshwater fish and contributing to the effective management of catchments.

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Freshwater Fish and Human Health
Appendix A

Fish as Bio-Monitoring Tools:
Development of the Fish Health Assessment Index (HAI)

by

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CONTENTS

FISH AS BIO-MONITORING TOOLS:- DEVELOPMENT OF THE FISH HEALTH ASSESSMENT INDEX (HAI)

INTRODUCTION

Various indices have been used to evaluate the condition or well-being of fish, including the relative conditions factor (Le Cren, 1951), relative weight (Wege and Anderson, 1978), the liver-somatic index (Heidinger and Crawford, 1977; Delahunty and De Vlaming, 1980), the gut index (Jensen, 1980), RNA-DNA ratios of liver and muscle (Bulow, Zeman, Winningham and Hudson, 1981; Devi, Gopal,& Gopal, 1991), and the visceral somatic index (Delahunty and De Vlaming, 1980; Adams, McLean and Parrotta, 1982; Diamond, Collins & Gruber, 1988). The latest trend however is to employ a holistic approach to assessment of overall fish health, in order to better evaluate environmental stressors. One such method to assess overall fish health is the Health Assessment Index (HAI).

The concept of using morphological or physiological characteristics to determine overall aquatic health lead to the development of a Fish Condition Profile (HCP). The Utah Division of Wildlife Resources developed fish health condition assessment procedures (HCP) for trout reared in state hatcheries and in feral populations (Goede, 1988). The method has been applied successfully in Utah since 1969 and has been used more recently to assess the general quality of juvenile anadromous salmonids (Novotny and Beeman, 1990).

The HCP is not intended to be diagnostic, but rather it is intended to provide a quick, simple means of judging the general health and condition of a group of fish (Novotny and Beeman, 1990). The key to the success of the procedure is the basic assumption that if fish are in good condition, the vital organs and other easily observed body structures will be in good condition (Novotny and Beeman, 1990). Once fish have been subjected to various stressors over an extended period, changes in organ appearance, morphology, or blood chemistry become apparent and can be recognised as serious departures from normal (Novotny and Beeman, 1990). If these stressors are left unchecked, the fish becomes increasingly susceptible to disease, and mortality may result (Novotny and Beeman, 1990).

The value of this system is in an accumulated database; single observations cannot be considered indicative of normality, or a deviation from normality, for a particular group of fish (Novotny and Beeman, 1990).

The HCP involves a series of simple, ordered observations and measurements of external characteristics, simple blood parameters, and external organs of a sample of twenty fish (Novotny and Beeman, 1990). For each fish, length and weight are recorded and condition factor is calculated; a sample of blood is drawn for determination of haematocrit, leucocrit, and concentration of blood plasma protein; morphological observations are made of the gills, pseudobranchs, thymus gland, and eyes; and finally, the body cavity is examined to evaluate the spleen, liver, hindgut, kidney, mesenteric fat, and bile in the gall bladder (Novotny and Beeman, 1990).

Means, standard deviations (Stdev), and coefficients of variation (CY = 100 X Stdev/mean) of lengths, weights, condition factors, and blood values are calculated and summarized individually. Scores assigned to each of the remaining observations are transformed into percentages or ratings and are included as components in the final autopsy summary for each group of fish. Deviations from the normal are considered indicative of some type of existing or developing problem within the population tested (Novotny and Beeman, 1990).

The HCP was designed in association with the autopsy-based condition assessment, developed by Goede and Barton (1990). This autopsy-based condition assessment was developed to meet the needs for a rapid, inexpensive, and easily used method for biologists to detect changes in the health of fish populations, early enough for corrective action to be taken (Goede and Barton, 1990). Like the HCP the autopsy system was not designed to be diagnostic or to solve specific problems related to fish health or the environment. Nor was the system designed as a substitute for other approaches or to meet all needs (Goede and Barton, 1990). The value of this system was that it provided a means of establishing a database for detecting trends in the health and condition of fish populations (Goede and Barton, 1990).

Use of the autopsy method requires several assumptions. (1) In fish under stress, tissue and organ function will change in order to maintain homeostasis; (2) if a change in function persists in response to continuing stress, there will be a gross change in the structure of organs and tissues; (3) if the appearance of all organs and tissues are normal according to the autopsy criteria, there is a good probability that the fish is normal; and (4) if the appearance of an organ or tissue system departs from the normal or from a control condition, the fish is responding to changes brought about by the environmental stressor (Goede and Barton, 1990).

Proper use of the method entails some further consideration. (1) The environmental stressor may be sufficiently severe that fish die before observable changes in structure or appearance occur; (2) there can be microscopic or histological structural changes without gross manifestation. A gross change may be predicted from histological change, but not the converse; (3) comparisons of stressed fish against normal or control conditions should be based on fish of the same age, strain, species, sex, and season; and (4) the individual expressing the condition in the sample must be representative of the population (Goede and Barton, 1990).

The major limitation of the necropsy or health profile method is that it does not provide quantitative results that are amenable to statistical comparison of data among sites, species, or years (Adams, Brown & Goede, 1993) and it was therefore modifed and refined to the necropsy-based approach to (1) provide a quantitative index so that statistical comparisons can be made between data sets; (2) include variables in the health index that reflect the degree of damage incurred as a result of environmental stressors; and (3) provide examples of the use of this index in different types of aquatic systems to demonstrate and validate its applicability (Adams *et al.*, 1993). These final modification made to the necropsy-based approach lead to the development of the Fish Health Assessment Index (HAl).

The necropsy method of Goede and Barton (1990) consists of 16 variables that can be grouped into the following categories: (1) three blood parameters (haematocrit, leukocrit, and plasma protein); (2) length, weight and condition factor; (3) percentage of fish with normal and abnormal eyes, gills, pseudobranchs, spleens, kidneys, and livers; and (4) index values of

damage to skin, fins, thymus, hindgut inflammation, fat deposits, and bile colour (Adams *et al.*, 1993).

Modifications made to the necropsy based approach by Adams et al. (1993).

Variable ranking:- To account for differences in severity of damage or level of effect, some variables of the HAl are assigned values of 10, 20, or 30, depending on the extent of the abnormality or observed damage (Adams *et al.*, 1993). Variables of the HAl that receive assigned values are fins, parasite loads, thymus condition, hindgut inflammation, skin condition, haematocrit and plasma protein levels (Adams *et al.*, 1993).

Calculation of the HAl:-. To calculate the HAl for each fish within a sample, numerical values for all variables are summed. The HAl for a sample population is then calculated by summing all individual fish HAl values and dividing by the total number of fish examined for that sample. A standard deviation and coefficient of variation is calculated (Adams *et al.*, 1993). The HAl for the sample population is composed of multiple HAl observations calculated for each fish within that sample. Therefore, statistical comparisons can be made among sample sites, sample times for the same site, and even species (Adams *et al.*, 1993).

South Afican situation

Avenant-Oldewage and Swanepoel (1993) have suggested the use of fish health as an indicator in South Africa. Subsequently the index proposed by Adams *et al.* (1993) has been applied with small modifications for local conditions through various studies by researchers associated with the Rand Afrikaans University. In the Olifants River System (Avenant-Oldewage, Oldewage and van Vuren, 1995) these projects made use of a variety of indicator species, including sharptooth catfish *Clarias gariepinus* (Marx, 1996; Watson, 2001), *Labeo spp.*(Luus-Powell, 1997), *Oreochromis mossambicus* (Robinson, 1996, Watson, 2001) and *Labeobarbus marequensis* (Watson, 2001). In the Vaal River System the index was evaluated on *Labeo umbratus* and *L.capensis* by Groenewald (2000), on *Clarias gariepinus* by Crafford (2000), on *L. umbratus, L. capensis, Cyprinus carpio, Labeobarbus aeneus and Lb. kimberleyensis* by Avenant-Oldewage (2001) as well as by Kotze (2002) on *L. capensis, L. umbratus* and *Lb. aeneus* from the Klip River. The Department of Water Affairs and Forestry has also incorporated the index in " Field Biosurveys and Integrated Ecological Assessment" (Kilian, 1996) and Kilian,Kleynhans, Du Plessis& Hoffman (1997).

The approach in the South African studies varied from the elaborate were all the parameters set by Adams *et al.* (1993) were evaluated and supplemented by complete analysis of endo and ectoparasites to more rapid assessment were fish were not killed and analysis were limited to external parameters which could be assessed from living specimens.

Olifants River

The studies in the Olifants River are summarized in the study by Watson (2001). She combined the data from the previous three studies (Marx, 1996; Robinson, 1996 & Luus-Powell, 1997) with the data sampled in the follow up study (Watson, 2001) in order to access a larger data set. All the parameters set by Adams *et al.* (1993) were taken into account and parasites were sampled and identified. The parasites were later grouped to represent ecto

and endoparasites. Data was collected for dam and river conditions and the studies were conducted in drought (1994) as well as flood (1996) conditions. In the studies a polluted as well as unpolluted sites were assessed. These were respectively Mamba (polluted) and Balule (unpolluted) in the Kruger National Park and Loskop Dam (polluted) and Bronkhorst Dam (unpolluted). The purpose was to determine whether any of the variables could be eliminated and it was therefore endeavoured to find the single or a combined set of variables which would most accurate indicate a difference between the sites.

Therefore, the data was subjected to logistic regression analysis to determine which of the parameters best distinguish between fish from the different localities. Before the logistic regression analysis was performed cross tabulation techniques and Chi^2 analysis was used to eliminate those variables which did not provide any significant difference between sample sites thereby limiting the amount of data required to develop a model using logistic regression analysis.

Parasite information was evaluated in three different manners, i.e. as proposed by Adams, *et al.* (1993), seperated into endo and ectoparasites and refined were the parasites received a graded score. Only two sample sites were assessed during drought and flood conditions, that is, Mamba and Balule in the lower Olifants River catchment area. Data collected from *C. gariepinus* and *0. mossambicus* were assessed respectively.

When a combined data set (variables collected for *C. gariepinus* and *0. mossambicus*) was evaluated during drought conditions, parasites and fins were the only variables selected for during assessment of the original HAl, Endo/Ecto Present Index and Refined Index respectively. When data collected for *C. gariepinus* was assessed on its own, parasites were again selected for, using all three index types respectively. Using *0. mossambicus* data the same three variables were selected, for all three index types namely, parasites, fins and liver respectively. Thus the only variable selected for using a combined data set and data collected for *C. gariepinus* and *0. mossambicus* separately were parasites.

Percentage classifications improved with modifications made to the original HAl, with the highest number of correctly classified fish being observed when the Refined Index was employed. This index distinguishes between endo and ectoparasites. Cross tabulation of variables excluded from the Endo/Ecto Present Index using a combined data set, indicated a relationship existed between ectoparasites present and skin. This relationship between ectoparasites and skin was also observed when data collected for *0. mossambicus* was assessed on its own for all three index types. *Lernaea* were observed on the skin of *0. mossambicus* causing an inflammatory response on the skin of affected individuals. Skin abnormalities are thus directly related to this ectoparasitic infestation, and consequently skin was excluded from the model to avoid repetition of results. WBC % was eliminated when the Refined Index was employed using the combined data set. A relationship appears to exist between excluded WBC % and skin, this suggests a link between immune response and damage found on the skin. *Lernaea* cause inflammation at the site of attachment, inflammation can be seen as a product of an immune response, which could explain the relationship, which appears to exist between skin and WBC %.

For data collected for *C. gariepinus*, eliminated during cross tabulation, a relationship appears to exist between all parasites and WBC %, when the original HAl model was employed. This suggests an immunological response exists, which may be due to parasite infections. Observed parasite infections were higher in *C. gariepinus* when compared to *0.*

mossambicus during drought and flood conditions, thus an observable immunological response would be expected when data collected from the control and more polluted river points are compared.

During cross tabulation comparisons using the combined data set and data for *C. gariepinus* and *0. mossambicus* separately, relationships also appear to exist between fins and skin, fins and WBC %, fins and liver, skin and WBC %, and between WBC% and endoparasites refined respectively, no positive link could be made to suggest that these relationships exist other than by chance.

When the models developed during drought conditions was employed on data collected during the 1996/1997 flood, the original HAl provided the highest correct classification of fish. The variable selected for throughout this comparison, using a combined data set, and data collected for *C. gariepinus* and *0. mossambicus* on the original HAl was employed. Variables excluded from the model showed only one possible relationship existed namely between all parasites and WBC % when the original HAl was employed and only when using a combined data set. The same trends were observed for the model developed for *C. gariepinus* during drought conditions also using the original HAl model.

As suggested in the above discussion, when cross tabulation and Chi^2 analysis was carried out on flood data in order to develop a model specific for flood conditions, blood variables and parasites were selected as being indicative of differences observed in fish caught at Mamba and Balule respectively. The new models developed during flood conditions indicated that WBC % and parasites, more specifically endoparasites in *C. gariepinus*, were most suggestive of differences between the fish sampled.

All parasites were again selected using *0. mossambicus* data separately, consequently the Endo/Ecto Present Index model failed for this fish species. Parasite numbers collected for this fish species were low during flood conditions, with no ectoparasites observed. Thus the all parasites variable selected for by the model for *0. mossambicus* data, can be considered comparable to the endoparasites present.

***C. gariepinus* .**

When compared to the model developed during drought conditions whereby both endo- and ectoparasites were selected, the effects of the floods on the Olifants River system becomes evident. During flood conditions ectoparasite numbers dropped significantly at Mamba and Balule on *C. gariepinus* and *0. mossambicus* respectively. This can be attributed to a decline in water quality observed at both sample sites, more specifically at Balule, with the removal of the purifying reed beds upstream with the resultant increase in particles in the water. Accurate observations of ectoparasite numbers are however not always possible. Sampling techniques, i.e. gill nets, whereby parasites leave the host when they become stressed, and the use of fishing rods, whereby hazardous terrain (crocodiles) does not always allow one to enter the water to collect the fish, may cause removal of ectoparasites before they can be counted. Endoparasites cannot escape their hosts, thus accurate assessments are made of infestation rates. During development of the flood models the Refined Index could not be employed, as the data set was not big enough. This was due to the overall decrease in ectoparasite numbers observed at both sample sites during the flood.

To summarise it was found that parasite numbers (endo- and ectoparasites) on their own could determine differences in fish sampled at Mamba and Balule, provided prevailing environmental conditions remained constant, as observed during drought conditions. Once fish are exposed to stressors in their environment, immunological response mechanisms come into play (WBC %). Parasites too continue to play a role during flood conditions. Changes observed in the model developed during flood conditions can be seen as a way of compensating for environmental stressors exerted on the different parasite groups, namely endo- and ectoparasites respectively, ultimately resulting in the elimination of ectoparasites from the model as an unreliable indicator of differences between sample sites. Therefore the only constant (variable repeatedly selected for) during drought and flood conditions and for assessments of data collected for *0. mossambicus* and *C. gariepinus* were endoparasites. It is thus suggested that endoparasite numbers and WBC % should provide the best overall assessment of fish condition in the Olifants River system, and should not be discarded from the HAl if variables are being eliminated in order to streamline the HAl evaluation procedures.

Similar trends were observed by Robinson, Hines, Sorensen and Bryan (1998) in the selection of blood variables and parasites, when determining overall fish health using logistic regression analysis. This author assessed the relationship among fish health, eukaryotic parasites, and bacterial and viral infections of endangered fish collected from two locations (Perkinsville and Childs) on the Verde River, Arizona, during February and June 1996 (Robinson *et al.*, 1998). Regression analysis on the calculated health assessment index, haematocrit and leukocrit, indicated that month, site, *Trichodina*, *Ichthyophthirius* (ich), *Ornithodiplostomum* and *Posthodiplostomum* (white grub), and the cestode I*soglaridacris hexacotyle* negatively affected the health of fish (Robinson *et. al.*, 1998) i.e. these variables best indicated degredation in fish condition, and thus provide the best assessment of differences between sample sites.

Correspondence analysis

Overlap only occurs between the polluted sample sites. Thus it can be suggested, that stress reactions on fish when exposed to pollutants are the same at different localities when exposed to the same pollution types, however, this distribution may also have occurred by chance. Only one grouping of variables was able to predict the spatial distribution at Mamba, namely, normal WBC %, abnormal plasma protein and endoparasite numbers respectively.

Grouping of variables for Loskop Dam provided a wider spatial distribution for this sample site. This indicates that the conditions observed at Loskop Dam are highly variable when compared to the polluted river point. This comparison possibly suggests differences observed between dam and river points with respect to exposure potential of fish to pollutants. Thus combinations of abnormal WBC % and plasma protein concentrations, as well as, abnormal WBC %, plasma protein concentrations and endoparasite numbers and normal WBC %, plasma protein concentrations and endoparasite numbers, indicate that fish were affected by the presence of pollutants to varying degrees at the dam site. Loskop Dam is the largest impoundment in the Olifants River system and the volume of water allows for variable exposure to pollutants, allowing for fish to be reported with a normal condition profile, even though one is dealing with a polluted site. This indicates the importance of gathering a representative sample of the population, before making final assessments about the health profile of fish in a specific impoundment. The importance of having no overlap between the control sites, or of control sites with polluted sites, indicates that any comparisons made

between control and polluted sites in the upper and lower catchment areas respectively, can be done knowing that differences exist.

Vaal River

In the studies on the fishes of the Vaal River various levels of analysis were employed. Groenewald (2000) applied the index in its original format to fishes collected from Vaal Dam, Abrahamsrus, Vaal Marina and Vaal Barrage and identified parasites that were visible to the naked eye. She mentions the presence of *Argulus, Lernaea*, unidentified nematodes and black spot. She records moderate to severe gill damage and no damage to external variables. Very high concentrations of abnormal livers were recorded (89% and 93% respectively) in *L. capensis* and *L. umbratus*. High plasma protein and haemaocrit levels were also recorded although the White cell counts were in the normal range. Only moderate parasite loads were recorded. She came to the conclusion that metal concentrations in the localities exceeded the TWQR for aquatic ecosystems and this could is likely the reason for the high Health index values recorded. The gills were pale although the water was well oxygenated. A correlation between the health values and the different localities was not establish.

In the study by Crafford (2000) on *C. gariepinu*s the index suggested by Adams *et al.* (1993) was employed and the parasites were identified in order to distinguish between endo and ectoparasites repectively. This data and data on other species collected from the Vaal Dam and Vaal Barrage was analysed and presented to the Rand Water Board (Avenant-Oldewage, 2001). This data was also subjected to logistic regression in order to determine which variable could be eliminated without a significant effect on the resolution of the index. *The following was found:*

During stepwise logistic regression analysis it became clear that various parameters classified the different species correctly. Small differences are visible as calculations were made to eight digits behind the comma. If nonsensical parameters such as weight and length are excluded from the stepwise logistic regression model in *Labeo capensis,* WBC were accurate in 98.3% of times when combined with season and fork length and in 80% when combined with fork length only. In *L. umbratus* the condition of the hindgut made it possible to distinguish between the different localities in 92.7% of cases. In *Lb. aeneus* it was effective when all parasites were combined in 97.4 of collected specimens. In *C. gariepinus* the inverted parasite index was accurate in 74.7% of cases and the predictive reliability could be enhanced by adding the Heamatocrit value as well as inverted parasite index in 76% of cases. It is deducted by Avenant-Oldewage (2001) that the application of the Health assessment index made it possible to distinguish, in the majority of cases, between the two localities. A difference in reliability for different seasons was only recorded in *L. capensis*. It is, however remarkable that the parameters that distinguished correctly in most instances are exactly the same ones that are excluded by Kilian (1996) in the document submitted to Dept. of Water Affairs.

In the most recently published study conducted by Kotze (2002) (conducted August 1997-February 1999). He sampled *Lb aeneus. L.capensis* and *L. umbratus* from seven localities in the Suikerbosrand and Klip River. The index proposed by Adams *et al.* (1993) as adjusted by Kilian *et al.* (1997) was employed whereby the condition of the skin, fins, eyes, opercula,

gills and number of ectoparasites are monitored. This method was selected to be able to release all fish specimens alive. He adjust the method by adding an additional grading for moderately affected organs and excluded the number of ectoparasites. He was able to distinguish between the sites in his survey and came to the conclusion that the opercula were seldom affected and should be excluded in future rapid surveys. The gills exhibited the highest amount of abnormality and he comes to the conclusion that the value of this organ as an indicator should not be understimated in environmental degradation studies. The second most sensitive organ was the skin.

Development of the original index

In the original guide (Adams *et al.,* 1993) the interpretation of colour for instance, creates a problem, as different evaluators will give different values to the same organ as their perception of colour differs, therefore a colour chart was developed (Watson, 2002). This chart was standardized and show the colours that can be expected to occur.

Adams *et al.* (1993) indicated that the index does not allow for identification of the source of environmental deterioration. Avenant-Oldewage (2001) emphasized that it should not be attempted to compare different environments to one another and that the index is valuable when it is used repetitively over a period of time in the same locality. The surveys should be done at the same time of year as breeding of fishes, flood conditions etc. influence the sensitivity of the index. The same species of fish should be used a test organisms. Late summer months proved to be ideal as floods are over, fishes terminated their breeding cycle and yet was still actively moving around, allowing gill net fishing to be successful.

Use of parasite data

Parasite data can be used to great effect in environmental management. The parasite fauna of migratory salmonids have been successfully used as biological tags, yielding information on fish movement and stock composition (Beverly-Burton, 1978; Frimeth, 1987; Bailey, Margolis and Groot, 1988; Bailey, Margolis and Workman, 1989; Bouillon & Dempson, 1989). In fact, parasites can provide information on varied aspects of salmonid biology, including mode of life, feeding habits, population dynamics and phylogenetic development (Urawa, 1989). More relevant to the current study, however, is the use of parasites as indicators of environmental health. Overstreet (1997) stated that “when an appropriate fish host is selected, analysis of its parasites offers a useful, reliable, economical, telescoped indication or monitor of environmental health”. After a short review of literature concerned with the use of parasites as pollution monitors, Rohde (1993) also concluded that parasites might be useful models for modeling pollution. He however feels that much more work is needed. Up to now biomonitoring of parasites have been conducted on a number of ecological scales, ranging from communities (Khan, 1987; Khan and Kiceniuk, 1988; Valtonen, Holmes & Koskivaara, 1997; Yeomans, Chubb & Sweeting, 1997) to ecological guilds (Bagge & Valtonen, 1996; Dusek, Gelnar & Sebelova, 1998) to genus level (Halmetoja, Valtonen & Taskinen, 1992; Khan, Barker, Williams-Ryan & Hooper, 1994). Parasite community composition in terms of endo- and ectoparasite numbers can be used as an indicator of heavy metal pollution (Avenant-Oldewage, 1994). Parasitological data are sometimes combined with histopathological data from the host (Overstreet, Hawkins & Deardorff, 1996), or in the case of the HAI, with gross tissue and organ anomalies.

In the original HAI (Adams *et al.* 1993) parasites are recorded as being absent or present. Ectoparasites are just as exposed to the environment as their host. It is thus assumed that poor

water quality will adversely affect ectoparasites to a greater degree than it would endoparasites (Avenant-Oldewage, 1994). From this assumption it follows that lower numbers of ectoparasites will correlate with a decrease in water quality. Higher numbers of endoparasites will thus indicate poor water quality and vice versa. Admittedly this is a massive generalization. In fact, Poulin (1992) states: "Thus, on the basis of toxic effects on fish, it is difficult to predict whether levels of parasitism in fish would increase or decrease following exposure to toxic pollutants in natural systems". As pollution-induced effect on levels of parasitism is superimposed on a significant natural variability in parasite prevalence and abundance (Siddall, Pike & McVicar, 1994) caution should be exercised in the interpretation of positive or negative correlations (McVicar, Bruno & Fraser, 1988). Also, increases or decreases of parasite numbers in response to decreasing water quality largely depend on the type of pollution (Lafferty, 1997).

This variability becomes clear when reviewing available literature (e.g. Skinner 1982; Thulin, Höglund & Lindesjöö, 1988; Khan, 1990). As a result single measures of communities of parasites (e.g. species richness) cannot be used as indicators of specific environmental change, because different taxonomic groups often respond in opposite directions (Lafferty, 1997). Some authors combine several measures of community structure to demonstrate differences in species composition related to water quality (Gelnar, Sebelova, Dusek, Koubkova & Juradja & Zahradkova, 1997). Lafferty (1997) states: "However, community-level monitoring will provide a detailed assessment of environmental change as long as analyses take into account the relative sensitivities of each group". Endoparasites can be seen as indicators of indirect stresses, while ectoparasites, being directly in contact with contaminants, are indicators of direct stresses (Gelnar *et al.,* 1997). Since parasites are associated with the HAI (meant to be a simple and rapid index), only general trends in parasite numbers are of interest. Trends like the one uncovered by Zharikova (1993) where the majority of ectoparasites exhibited a decrease in numbers in response to industrial (heavy metal) and domestic pollution.

Internationally the use of parasites as indicators of pollution is under scrutiny. Most of these studies concentrate on the effect of organic pollution. It is furthermore known that the presences of specific groups of parasites are indicative of a variety of pollutants. For instance *Gyrodactylus* infections increased with increase in exposure to crude oil, water soluble oil fractions and sewage (Khan and Kiceniuk, 1988; Khan and Thulin, 1991 and Yeomans, Chubb and Sweeting, 1997). Similarly a decline in numbers were observed when these parasites were exposed to kraft mill effluent however the prevalence and intensity decreased when fish were exposed to kraft mill effluent which included chlorine (Thulin *et al.,*1988;Khan, Barker, Williams-Ryan and Hooper, 1994; Bagge and Valtonen, 1996). Zharikova (1993) revealed a decrease in numbers following exposure to heavy metals in *Gyrodactylus*, Digenetic Trematode larvae, leeches and the copepod *Ergasilus sieboldi.*

Riggs and Esch (1987) found an increase in prevalence and abundance of cestodes from polluted fish was higher than that of fishes from heavy metal unpolluted water bodies. This finding is further supported by Sures, Siddal and Taraschewski (1999) who indicated that an acanthocephalan *(Pomphorrhynchus laevis*) present in the intestine of the host could absorb 2700 times the amount of lead and 400 times the level of cadmium than what was present in their host muscle tissue. Furthermore *Monobothrium wageneri* (cestode) contained 150 times more lead and 40 times more cadmium than the muscle of their host, tench (Sures, Taraschewski and Rocicki, 1997). Pascoe and Cram (1977) published results were host fishes were exposed to different concentrations of metals after being infected with plerocercoids of

cestodes. The parasites remained alive even after their hosts died. Crafford (2000) as well as Watson (2001) discuss these matters in much detail and Lafferty (1997) presents a detailed summary of existing knowledge.

Furthermore, pollutants may affect the immune system of the hosts, which in turn increase the outbreak of parasitic diseases.
Sharptooth catfish, although described as “not harbouring any more or any less parasites than other fish species” (van As and Basson, 1988), play host to a great variety of endo- and ectoparasites. This makes them ideal for use with the Health Assessment Index. Catfish also proved to be a good indicator species in other respects. It is the most widely distributed fish in Africa (Skelton, 1993) and accumulates a wide variety of metals in various tissues (Marx, 1996). Fish species near the top of the food chain are generally regarded as representative indicators of overall system health (Adams *et al.*, 1993). Catfish are omnivorous, incorporating a wide variety of aquatic and terrestrial foodstuffs through hunting and scavenging (Skelton, 1993; Skelton, 2001)). They are generally considered the most hardy freshwater fish species in South Africa (Skelton, 1993, van As and Basson, 1988), but tissue and organ anomalies resulting from environmental stress can be observed (Marx, 1996). This makes them good indicators of chronic environmental stress, enabling them to reflect “accumulative” effects of both past and recent water quality conditions.

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Freshwater Fish and Human Health
Appendix B

A Reference Guide for the Determining the Human Health Risks of Consuming Freshwater Fish in South Africa

Protocol for the Assessment of Fish Health

User's Manual

by

Annemarié Avenant-Oldewage

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PROTOCOL FOR THE ASSESSMENT OF FISH HEALTH

USER' S MANUAL

1. INTRODUCTION

A manual for the evaluation of fish health through a necropsy-based method was suggested by Adams, Brown and Goede (1993). This index is in use in the United States of America for the determination of the effect of paper mill effluent on fish health. The use of this index allows for a number of assumptions (Goede and Barton, 1990). These are:

1. In fish under stress, tissue and organ function will change in order to maintain homeostasis.
2. If a change in function persists in response to continuing stress, there will be a gross change in the structure of the organs or tissues.
3. If the appearance of all organs and tissue are normal according to necropsy criteria, there is a good probability that the fish is normal.
4. If the appearance of an organ or tissue system departs from the normal or from a control condition, the fish is responding to changes brought about by the environmental stressor.
5. The environmental stressor may be sufficiently severe that fish die before observational changes in structure or appearance occur.
6. There can be microscopically or histological structural changes without gross manifestation. A gross change may be predicted from histological change, but not the converse.
7. Comparisons of stressed fish against normal or control conditions should be based on fish of the same age, species, strain, sex and season.
8. The individual expressing the condition in the sample must be representative of the population

This manual has been written as a preliminary guide to performing biological monitoring and is based on the health assessment of any fish species living in South African waters. It has been tested in locations in the Olifants River in the Limpopo River System and in the Vaal River System. Procedures for the initial testing of the HAI in the field were followed using the field guide set out by Klemm *et al*. (1992) and this manual serves to clarify the index. The interpretation of colour for instance, creates a problem, as different evaluators will give different values to the same liver as their perception of colour differs, therefore a colour chart is added (Avenant-Oldewage, 2001).

Furthermore the advantages and disadvantages of this method is summarized and a table lists the field and laboratory equipment used during practical work, as well as the procedures used when determining the HAI in field and laboratory. In addition, it highlights general observations made during the testing of the HAI together with recommendations, which will benefit subsequent users of the manual

There has long been a need for easier and rapid methods of biological monitoring of water quality. The South African Scoring System, Version 4 (SASS 4), has been used to fill this niche partly and this system where invertebrates are used as a guide to performing biological monitoring has earned a place as index reliable under specified conditions. The HAI index

should be evaluated in a similar way. Using these two and other biological indexes simultaneously should increase the value of all.

Minor updating may become necessary as the method is tested and developed further in additional biogeographic regions. In the meantime, it can be regarded as a useful working document.

Advantages of this method

- fish communities generally include a range of species that represent the variety of trophic levels and include food particles from the aquatic as well as terrestrial environment,
- fish are relatively easy to identify, (compare to invertebrates for instance)
- the general public can relate to statements about conditions in fish communities,
- both acute- and stress effects can be evaluated,
- fish are present in all but the most polluted rivers,
- the life-span of fishes are normally a number of years therefore their condition could give an indication of long term effects on a system,
- fish integrate the effects of many biotic and abiotic variables in the ecosystem, therefore, the condition of their external features, blood parameters and the appearance of their internal organs reflect whether they are in harmony with their environment,
- this method gives a rapid indication of the health status of a selected environment,
- the Health Assessment Index (HAI) is a quantitative index that allows statistical comparison between different water bodies and,
- although the exact cause of pollution cannot be assessed, the HAI is useful in assessing first level problems in the health profile of fishes.

Limitations of this method

- fish collection is time consuming and laborious,
- the method does not identify the nature of chemical changes (this is, in general, a limitation of most biological monitoring systems),
- too small sample sizes (less than 20 specimens) may obscure results (this is, in general, a limitation of most biological monitoring systems).
- biased results may be obtained if different people use the index
- experience may be needed for some procedures (this can be gained during a morning workshop)

2. EQUIPMENT AND MATERIALS

2.1 Field equipment

See Table 1 for the list of equipment necessary for the evaluation of the health assessment index under field conditions.

Table 1: List of equipment used during evaluation of Health Assessment Index in the field

APPARATUS	GLASSWARE
• microhaematocrit centrifuge • microhaematocrit reader / ruler • centrifuge for blood vials • petri-dish (±10) • 5 ml dropper (±5) • critoceal clay to seal haematocrit tubes • "collar needles" • disposable syringes • disposable needles (size 22G X 1¼) • metric scale to weigh individual fish • fish measuring board • tagging gun with tags (type of gun used to attach labels to clothes works well with water resistant labels) • dissection microscope • generator to create electricity for the oxygen pump, microscope and centrifuge • oxygen pump to aerate water in holding tank • gill nets 70, 90, 110 and 130 mm mesh size) and hand lines • angling rods and bait • absorbing paper towel • nail brush and ammonia liquid* for cleaning of dissection boards and equipment	• microhaematocrit tubes • 5 ml "vac-u-test" blood vials (rinsed with an anticoagulant*) • glass micro slides • sample bottles with lids

SOLUTIONS	DISSECTION INSTRUMENTS	MISCELLANEOUS EQUIPMENT
• heparin solution (5000 units per milliliter) • EDTA solution (3% ethylene diamine tetra-acetate solution into 0.1% NaC1 in distilled water)** • saline solution (0,1% sodium chloride in distilled water) • absolute methanol to fix blood smears	• bone scissors • sharp and blunt point stainless steel dissection forceps • small, fine point stainless steel dissection forceps ("Dumont tweezers") • scalpel with spare blades • dissecting board	• buckets and tubs to handle fish • table • chair/s • ice/ice packs

APPARATUS	GLASSWARE	SOLUTIONS
• Boehringer Mannheim Test-Combination kit (D-68298) • spectrophotometer • compound microscope	• pipette (0.1 ml and 5 ml) • cover slips • Entellan mounting medium	• Giemsa's stain solution (Saarchem cat nr. 264 50 20)

- **Ammonia liquid** is used to clear equipment from fatty remains of dissections. A cleaning agent with similar properties could replace it.

* ***Anticoagulant** EDTA (3% ethylene diamine tetra-acetate solution into 0.1% NaC1) retards clotting by chelating calcium ions required for prothrombin conversion to thrombin, is used for *Clarias gariepinus* or heparin (5000 units per milliliter which acts as an antithrombin for all other species. Vials can be obtained from suppliers.

3. PROCEDURE

3.1 Fieldwork

3.1.1 Sampling and collection of fish

1. Sample 20 fish of each species with gill nets, hand lines or angling rods. It is important that the sample should be as uniform as possible to allow for comparison.
2. Examine fish (entire body surface, fins, gill cavity and buccal cavity) individually for mobile ectoparasites as soon as retrieved.
3. Record number of parasites found (Table 2) and mark fish with a tag by inserting the needle of the tagging gun into the lateral muscle of the fish. Do not insert the label through a fin, as this tends to tear out while the fish are kept in the holding tank.
4. Place fish in holding tank into which air is bubbled with a pump.

3.1.2 Sampling and reading of blood samples

1. Prepare syringe by inserting ± 0.1 ml anticoagulant into each vacutainer. EDTA-solution for *Clarias* spp.. and for all other fish species, rinsing the entire length of the vacutainer by turning it upside down. (vacutainers pretreated with anticoagulant are available on the market at a slightly higher prize)
   - place fish horizontally on dissection board
   - cover eyes with damp cloth to minimize stress
   - draw ±1 ml blood from the dorsal aorta by inserting collar needle just below the lateral line. Insert the vacutainer into the needle and move the needle further down or up until blood start filling the vacutainer (This procedure needs assistance at first) (See Fig 4E).
   - Label the vacutainer.

2. Prepare blood smear slide as shown in Fig. 1.
   - Remove the lid from the vacutainer and draw a drop of blood from the vacutainer with an ordinary syringe and needle.
   - place a drop of blood about 1 cm from the edge of a microscope glass slide
   - apply a second slide just in front of the drop, hold the slide at an 45º angle and wait until the blood has spread across the width of the slide
   - push the slide smoothly forward to spread the smear in order to obtain a tongue shaped smear. Take care not to push the top slide in the wrong direction, which would result in the slide spreading over the blood film, instead “pull” the blood film.
   - After slide is air dried, preserve blood by dipping the slide in absolute methanol (Failure to do so at this stage will cause red blood cells to burst when they are stained). Keep the methanol container closed at all times as methanol absorbs water from the atmosphere and the concentration will therefore be reduced. The resulting lower concentration will not fix the cells and the cells will therefore burst as a result of osmotic shock when it is attempted to stain them.
   - Leave to air-dry, mark the slides with a number corresponding to the number on the fish tag.

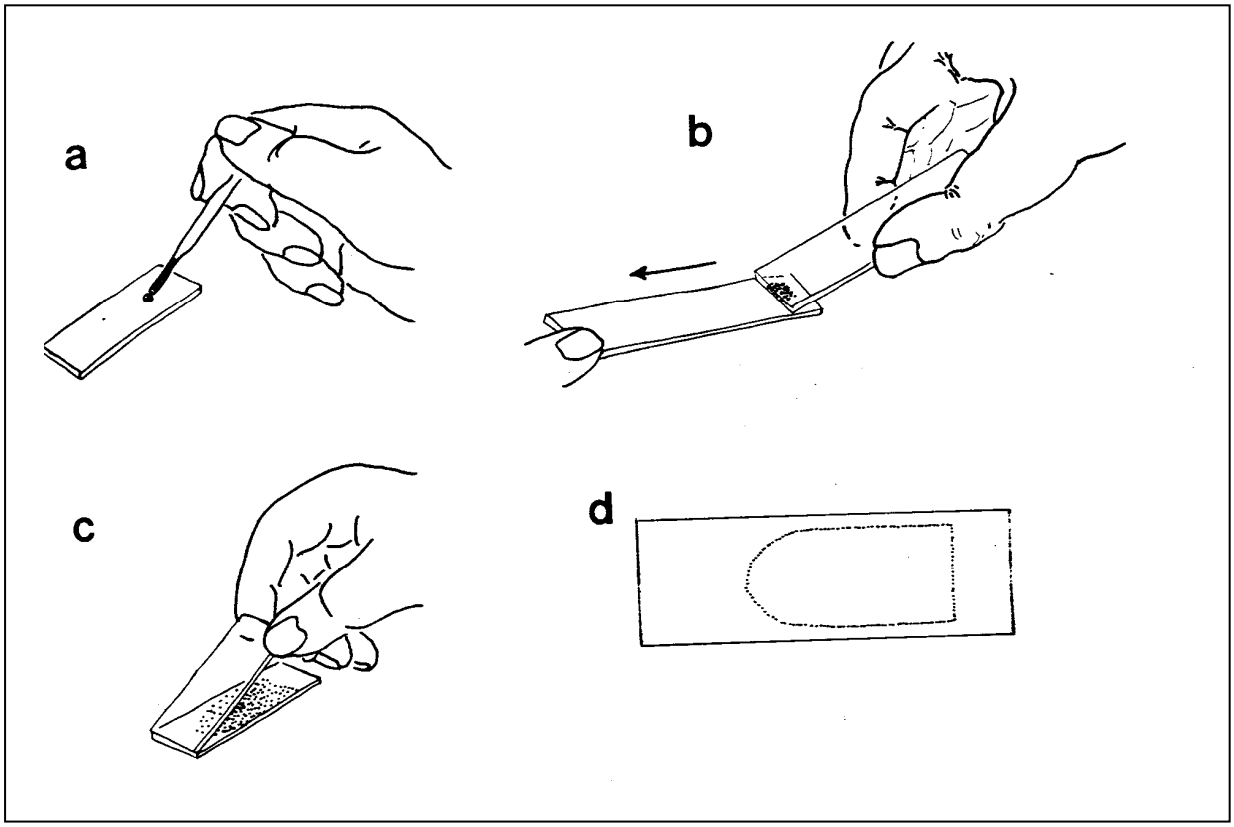

Figure 1. Illustration of the correct procedure to prepare a blood smear.

3. Place remainder of blood immediately on ice until ready to determine haematocrit.

4. Determine haematocrit as follows:
   - fill microhaematocrit tubes with blood by inserting one end into a blood vile, capillary action will "suck" blood into the microhaematocrit tube. Remove the microhaematocrit tube and
   - plug one end with "critoceal" clay
   - keep the tubes in order and place in numbered slots in microhaematocrit centrifuge
   - centrifuge for three minutes (15 000 revolutions per minute)
   - measure haematocrit by reading both the total blood volume and the packed red blood cell volume and the buffy layer using the microhaematocrit tube reader or a ruler. If a ruler is used the red blood cell reading (mm reading) is expressed as percentage of the total reading (from the bottom to the top of the plasma (mm reading) and the WBC count/leucocrit ("buffy layer" (mm reading) as a percentage of the total reading (mm reading).

5. Obtain blood plasma for blood protein analysis:
   - place blood vials (vacutainer) containing the remainder of the whole blood into centrifuge
   - centrifuge for 10 minutes at 3000 revolutions per minute
   - draw blood plasma (top layer) (see Fig 2b) into dropper and place in 5 ml sampling containers
   - freeze blood plasma if protein analysis in laboratory is not following immediately.

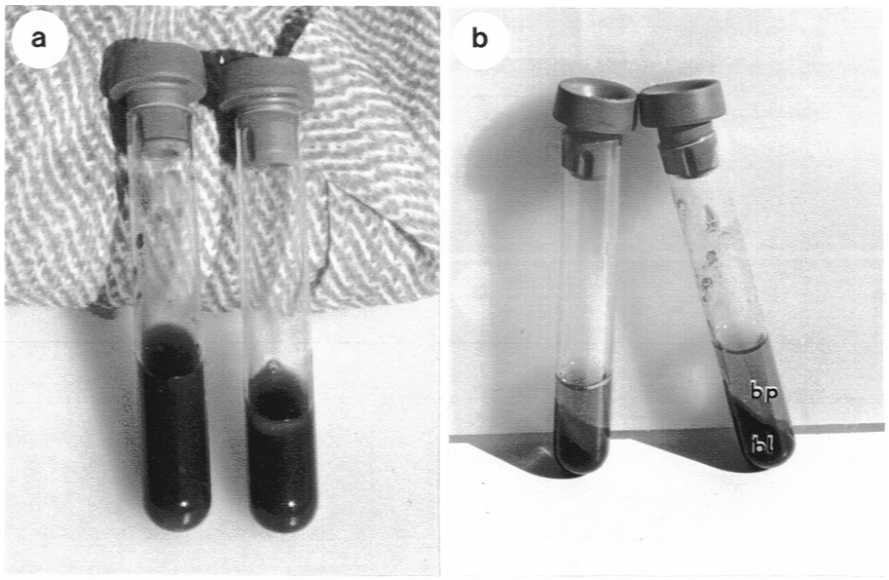

Figure 2. a Whole blood in blood vials; **b** Whole blood following centrifugation to show the clear blood plasma (bp) separated from the packed cells(bl).

3.1.3 Length and weight measurements

The length (mm) and the weight (g) of the fish are measured only after the blood has been drawn. In doing so, the period of handling stress is reduced before blood sampling, which minimizes changes in blood chemistry. Measure the total length in species where the tail fin is undivided and both the total length and fork length in other species.

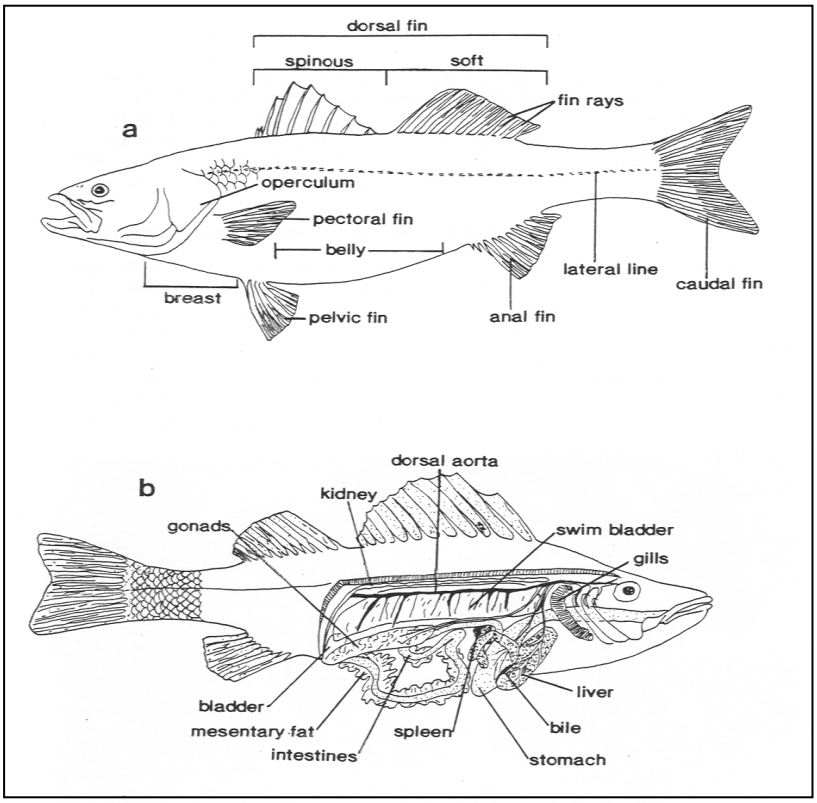

Figure 3. Lateral view of a spiny-rayed bony fish to show the morphology **a)** external features; **b)** view of a dissected fish to show the position of organs

3.1.4 Examination of fishes

1. Record all ectoparasites (Table 2). Be aware that some parasites are mobile (identification of parasites can be done with the aid of Fig. 5).
2. Examine fish externally (Fig. 3a) using the necropsy-based system (Tables 3&4).
3. Remove gills and place in petri dish. Cover gills with river or dam water to prevent dehydration.
4. Note gross pathological changes in the colour but also be on the lookout for parasites. A stereomicroscope may be useful and enhance chances of finding parasites. Parasites may be preserved in 70% ethanol.
4. Examine gills for parasites/cysts (Fig. 5) with the aid of a dissection microscope.
5. Dissect fish by opening the belly. Insert the scissors into the anus and cut towards the head. This allows for easy identification of an inflamed hindgut. See Fig. 5.
6. Examine fish internally (Fig. 2b) and assign designated characters to the organs as indicated in the necropsy-based system (Tables 3&4). Characteristics allowing for confusion due to differences in interpretation should be evaluated with the aid of the colour chart in Fig. 6. This chart was complied using standard colours in the Plascon Expressions range of paints. The code for each colour is provided and duplication should preferably by acquiring a set of colour charts from a paint supplier. Duplication with photocopiers and computer driven printers does not allow accurate reproduction. It is advised that the colour chart be covered in plastic, thereby allowing for the person applying the index to place a piece of the organ under scrutiny on the chart in order to compare the colour. This application allows for accurate repetition of result even when using different people applying the index.
7. Remove the intestine and place into a petri-dish containing saline. Record endoparasites from the body cavity, such as helminths and larval nematodes (identification of parasites can be done with the help of Figs. 4&5).
8. Cut the intestine open with a pair of scissors. In some species it is possible to tear the intestine apart by using two tweezers inserted into the lumen and pulling it apart. **Record all observation on table 3 (field observations)**

3.2 Laboratory work

3.2.1 Determination of plasma protein content of the blood

A Boehringer Mannheim Test-Combination kit (D- 68298) or a field determination method can be followed:

- preparation of the plasma samples using a Boehringer Mannheim Test-Combination kit (D-68298); the procedure outlined in the package insert must be used
- following preparation, place samples in a spectrophotometer, read at a wavelength of 546 nm and calculate absorption values

Another method for determining plasma protein, which is done in the field, is documented by Klemm *et al*. (1990).

3.2.2 *Determining white blood cell counts*

1. Stain fixed blood-smeared microslides with concentrated Giemsa solution, for approximately 5 minutes, rinse in water and allow drying for 24 hours.

2. Mount stained microslides with cover slips, using "Entellan" as the mounting agent (Cat. No. 1.07961).
3. With the aid of a compound microscope count both red and white blood cells (WBC) at 1000 x magnification, repeat on five randomly chosen places on the slide and determine the mean percentage WBC. A computerised microscope with the appropriate software can do be used to automate this task.
4. Record blood parasites on Table 2 if present. See Fig. 5A for identification.

3.2.3 *Calculation of fish health assessment*

1. Replace field observation values with HAI score values as indicated in Table 4 and add the values obtained through laboratory analysis. Calculate the HAI for each fish summing the numerical values for all variables (Table 4 last column).
2. Calculate the HAI for a sample population by summing all individual HAI values and dividing by the total number of fish examined for that sample (mean).
3. Calculate the standard deviation for each sample as follows:

$$SD = \frac{\sum_{i\text{-l}}^{N} (Vi - X)^2}{N\text{-i}}$$

N = number of fish per site
X = average index for each site
Vi = index value for fish i

4. Calculate the coefficient of variation as follows:

$$CV = 100 \times SD/X$$

3.2.4 *Condition factor (CF)*

Calculate condition factor for each fish as follows:

$$CF = \frac{W \times 10^5}{L^3}$$

W = weight
L = length

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KLEMM, D.J., STOBER, Q.J. & LAZORCHAK, J.M. 1992. *Fish field and laboratory methods for evaluating the biological integrity of surface waters.* U.S. Environmental Protection Agency. Cincinnati, Ohio. pp 239-288.

Table 2. Record card to document parasites observed

FISH HEALTH ASSESSMENT RECORD

Project Number: **Sampling date and time:**

LEVEL 3 SURVEY

Site name/ Number:_____

Province:_____ **GPS Coordinates:_____**

Water body Name:_____ **Water body Type:_____**

Sample type: (bottom feeder, predator): _____

Species Name:_____

Composite Sample #:_____ Replicate No:_____ No. of individuals: _____

Table 2 continued

PARASITES	FISH NUMBER																				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Epitheliocystis *																					
Larval nematodes (in body cavity)																					
Adult nematodes (in gut)																					
Unidentified monogeneans																					
Diplozoon sp.																					
Larval cestodes																					
Adult cestodes																					
Lamproglena sp.																					
Lernaea sp.																					
Protozoa																					
Leeches																					
Unidentified cysts*																					
• Gill filaments																					
• Gill arches																					
• Pseudobranch																					
• Visceral cavity																					
• Fins																					
• Muscle																					
• skin																					
Blood parasites																					

* cysts expressed as a percentage referring to the percentage of the organ covered by the cysts

Table 3. Record card used in the field to document the condition of fish health variables given score values from the necropsy based system represented in this table

FISH HEALTH ASSESSMENT RECORD

Project Number: **Sampling date and time:**

LEVEL 3 SURVEY

Site name/ Number:_____

Province:_____ **GPS Coordinates:_____**

Water body Name:_____ **Water body Type:_____**

Sample type: (bottom feeder, predator): _____

Species Name:_____

Composite Sample #:_____ Replicate No:_____ No. of individuals: _____

Table 3 continued Field observation value (Field code from Table 4)

Fish #	Lgth (mm)	Wght (g)	Sex	Eyes	Skin	Fins	Oper cula	Gills	Pseudo-branch	Thymus	Mesen-teric fat	Liver	Spleen	Hindgut	Kidney	Bile
1																
2																
3																
4																
5																
6																
7																
8																
9																
10																
11																
12																
13																
14																
15																
16																
17																
18																
19																
20																

Table 3 continued Laboratory value substituted from field observation (see Table 4) and laboratory observations

Fish #	Eyes	Skin	Fins	Oper cula	Gills	Pseudo-branch	Thy-mus	Liver	Spleen	Hindgut	Kidney	Blood values				Para-sites	Total value/ fish
												Hct %	Prot	Lct	WBC		
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	
11																	
12																	
13																	
14																	
15																	
16																	
17																	
18																	
19																	
20																	
TOTAL VALUE FOR SURVEY																	

Table 4. Detailed descriptions of various rankings allocated to variables for the necropsy based system (Goede and Barton, 1990; Klemm *et al.*, 1992, Avenant-Oldewage, 2001)

EXTERNAL VARIABLES	DETAILED DESCRIPTION	Field Code	HAI score value
Eyes			
normal	no aberrations evident (good "clear" eyes)	N	0
exopthalmia	swollen, protruding eye. E1- one eye; E2 – two eyes	E1/ E2	30
haemorrhagic	bleeding in the eye	H1/H2	30
"blind"	opaque (dull) eyes	B1/B2	30
"missing"	eye missing from the fish	M1/M2	30
other	any manifestations which do not "fit" the above	OT	30
Skin			
normal	no aberrations evident	0	0
mild skin aberrations		1	10
moderate skin aberrations		2	20
severe skin aberrations		3	30
Fins			
no active erosion	normal appearing fins or lesion healed	0	0
mild active erosion	active erosion with no haemorrhage or secondary infection evident	1	10
severe active erosion	active erosion with haemorrhage and/or secondary infection evident	2	20
Opercula			
normal	no shortening; gills completely covered	0	0
mild shortening	very small portion of the gill exposed	1	10
severe shortening	considerable portion of the gill exposed	2	20
Gills			
normal	no apparent aberration in gills	N	0
"frayed"	erosion of tips of gill lamellae; "ragged" look	F	30
"clubbed"	swelling of the tips of the gill lamellae	C	30
"marginate"	gill with a light discoloured margin along the distal end or tips of the lamellae of filament	M	30
"pale"	gills are definitely very light in colour	P	30
other	any observation, which does not fit above. *Take special note of the presence of mucous on the gills*	OT	30
Pseudobranch			
normal	the normal pseudobranch is quite "flat" or even concave in aspect and displays no aberration	N	0
swollen	convex in aspect	S	30
lithic	mineral deposits in pseudobranchs, manifested by the appearance of white, somewhat amorphous spots or foci	L	30
swollen and lithic	lithic pseudobranchs are often also swollen	S&L	30
inflamed	appear "red", includes haemorrhage and any other causes of redness	I	30
other	any manifestation observed in pseudobranch which is not mentioned above	OT	30

Table 4. (Continued)

EXTERNAL VARIABLES	DETAILED DESCRIPTION	Field Code	HAI score value
	Thymus		
no haemorrhage	appears to be in normal condition	0	0
mild haemorrhage	a few red spots or petechial haemorrhages evident, many pin point size	1	10
severe haemorrhage	many "pin point" haemorrhages evident with some of them coalescing	2	20
	Mesenteric fat		
	no fat deposited around the pyloric caeca or anywhere in the visceral cavity	O	-
	slight, where less than 50% is covered with fat	1	-
	50% of each caecum is covered with fat	2	-
	pyloric caeca are completely covered by a large amount of fat	3	-
	Liver		
	normal, good solid red colour	A	0
	lighter red than A (normal)	B	0
	"fatty" liver, light tan colour, such as "coffee with cream	C	30
	nodules in liver i.e., white mycobacterial cysts and incipient nodules	D	30
	focal discolouration	E	30
	colour change in the whole liver	F	30
	gross aberrations which do not fit the above, e.g. grey mottling, small sizes	OT	30
	Spleen		
black	very dark red colour	B	0
red	red colouration	R	0
granular	granular or "rough" appearance	G	0
nodular	contains fistulas or nodules of varying sizes, often cysts, such as mycobacterial infections	NO	30
enlarged	significantly and noticeably enlarged	E	30
other	gross aberrations which do not fit the above, e.g. mottling, small sizes	OT	30

Table 4. (Continued)

INTERNAL VARIABLES	DETAILED DESCRIPTION	Field Code	HAI score value
	Hindgut		
no inflammation	no inflammation or reddening occurs	0	0
slight inflammation	slight inflammation/reddening	1	10
mild inflammation	Mild inflammation	2	20
severe inflammation	considerable, severe inflammation/reddening	3	30
	Kidney		
normal	firm dark red colour lying relatively flat in the visceral cavity	N	0
swollen	enlarged or swollen wholly or in part	S	30
mottled	ottled or "patchy" appearance ranging from scattered patches of grey to total grey discolouration	M	30
granular	may have granular appearance and texture	G	30
urolithiasis	this condition is known as nephrocalcinosis and involves deposition of white or "cream colour" amorphous mineral material in the tubules of the kidney	U	30
other	any aberration which do not fit the above	OT	30
	Bile		
	yellow or straw colour, bladder empty or only partially full	0	-
	yellow or straw colour, bladder full, distended	1	-
	light green to "grass" green	2	-
	dark green, dark blue-green	3	-
	Blood haematocrit value		
Within normal range	30-45%	0	0
Above normal range	>45%	1	10
Below normal range	19-29%	2	20
Below normal range	<18%	3	30
	Blood plasma protein value		
Within normal range	30-69 mg/dL	0	0
Above normal range	70 mg/dL	1	10
Below normal range	< 39 mg/dL	2	30
	Leukocrit value		
Within normal range	< 4%	0	0
Outside normal range	≥ 4%	1	30
	White blood cell count		
Within normal range	< 4%	0	0
Outside normal range	≥ 4%	1	30
Parasites			
No parasites observed		0	0
ectoparasites observed	1-10 parasites		10
	11-20 parasites		20
	>20 parasites		30
endoparasites observed	<100 parasites		10
	100-500 parasites		20
	501-1000 parasites		30
	>1000 parasites		
Sex			
male	observation of testes	M	-
female	observation of ovaries	F	-

Table 5: Review of problems experienced with the health assessment during the present study. Included are recommendations associated with these problems

Problems experienced	Recommendations
The sample size of suitably large fishes required for this study (20) was difficult to obtain, especially during the winter months. The only fish collected abundantly was *Labeo* spp. These are, however not the most reliable species as was proven by the study by Watson (In press)	Collection techniques should be reconsidered. Another alternative is the introduction of cages similar to those used in the North Sea for Salmon farming. The cages could be stocked with fingerlings and submerged in a location were hosts would be exposed to food. In the case of *C. gariepinus* the cage should allow for air breathing.
Distribution of fish species differs in rivers and dams. These should therefore not be used alternately to collect fishes.	Conduct surveys in the same locality over time.
During hotter days, blood clotted rapidly. Clotting affect the filling of hamatocrit tubes. Blood smears are also affected, the red and white cells tend to separate. Separation of plasma could be inhibited.	Keep blood at all times on ice and conduct analysis as soon as possible- the maximum time lapse should be 24 hours.
Cappilary tubes breakages occur.	Take care when transporting cappilary tubes by packing them in sponge rubber to prevent invisible cracks to lead to breakages when the tubes are centrifuged.
Opinions differed when assigning different field codes to variables, i.e. colour and state of the liver, bile etc	Use the colour chart- taking care to duplicate it by using the original colour charts from a paint supplier.
The following variables created confusion	
Eyes Missing eyes could be a result from predation **Fins** Other factors could have an influence such as struggling in the nets. **Pseudobranchs** Not present in most species. **Liver** Variations in interpretations may occur. **Thymus** Not visible in mature fish **Bile** Information not used in calculation of Health index, but these values give an indication of the amount of time since the last meal has been taken	Predation should not occur on all specimens in the sample. It is important to note whether the lesions are fresh. Exclude in species where it is absent. Use the colour chart Omit for mature fish. Make a note