A METHODOLOGY FOR UNDERTAKING FRESHWATER FISH CHEMICAL CONTAMINANT SURVEYS FOR HUMAN HEALTH RISK ASSESSMENT

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SUMMARY

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. Although the consumption of fish is generally beneficial to people (good source of protein, vitamins, omega fatty acids and basic minerals) consumers of fish are potentially at risk as fish have the potential to bioaccumulate contaminants from the aquatic environment that pose carcinogenic, genotoxic and non-carcinogenic health risks to them.

As a result of the potential health risk associated with the consumption of chemical contaminated non-commercially caught fish, the United States of America Environmental Protection Agency (US EPA) has developed a series of four guiding documents for issuing fish consumption advisories. The fish consumption advisories are designed to reduce the risk to fish consumers by providing information that would lead to the voluntarily restriction of fish consumption to levels that pose limited, if any risk. 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 based fish consumption limits for humans. To address this limitation the general objective of this dissertation is to develop a generic methodology 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 fisherman. Furthermore, the methodology would also give guidance to surveys investigating the chemical contamination of fish for ecosystem health assessment programmes.

The fundamentals of the methodology 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 methodology, 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.

The study on the Vaal River Barrage Reservoir and the Klip River indicated that there is potential metal health risks (mainly nickel related) associated with the daily consumption of fish from this system. The finding of this study therefore supports the viewpoint that by monitoring the chemical contaminant levels in freshwater fish and applying a risk-based approach valuable information regarding the possible health risk to the consumers of fish (especially to recreational and subsistence fisherman) can be obtained. These surveys also identify areas in the aquatic system where aquatic life and especially fish have unacceptable chemical contaminant levels due to anthropogenic activities in the catchment. This information can thus be used in catchment management programmes and thereby contribute to the management of the catchments in South Africa.

From the foregoing it is evident that by following and implementing the methodology proposed in this dissertation a major contribution would be made towards the protection of the consumers of freshwater fish as well as to the protection the of freshwater aquatic environment. These studies are therefore essential for achieving the ultimate goal of ensuring that the fish populations are fit for present and future human consumption. As the Department of Water Affairs and Forestry is the custodian of freshwater systems in South Africa, the monitoring of chemical contaminant levels in fish according to the proposed methodology should be implemented and managed by the Department in collaboration with other governmental organisations and the Catchment Management Agencies.

OPSOMMING

OPSOMMING

In Suid Afrika kan die besoedeling van varswaterakwatiese sisteme aan puntbronne (afvalwatersuiweringswerke- en industriële uitvloeisels) en diffuse oppervlakwater-afloop (landbou, mynbou en stedelik) toegeskryf word. Bogenoemde menslike aktiwiteite kan onskuldige mense, asook ander vorms van lewe, aan skadelike kontaminante blootstel as dit sonder die inagneming van die moontlike gesondheids- en omgewingseffekte vrygestel word. Studies het bevind dat mense aan oppervlakwater-kontaminante gedurende ontspannings-aktiwiteite, die drink van water en die eet van gekontamineerde voedsel blootgestel word. Alhoewel die eet van vis gewoonlik voordelig vir die mens is (bron van proteïne, vitamine, omega-vetsure en basiese minerale) kan daar ook 'n gesondheidsrisiko wees aangesien visse die vermoë het om besoedelingstowwe van die omgewing te bioakkumuleer. Hierdie besoedelingstowwe kan karsinogeniese, genotoksiese en nie-karsinogeniese gesondheidsrisiko's vir die mens inhou.

Aangesien daar 'n moontlike gesondheidsrisiko bestaan met die eet van chemies gekontamineerde visse wat nie-kommersieël gevang word, het die 'United States of America Environmental Protection Agency' ('US EPA') 'n reeks van vier dokumente gepubliseer om leiding vir die onwikkeling van visverbruikadvies inligting te gee. Die visverbruikadvies inligting is ontwerp om die gesondheidsrisiko verbonde aan die eet van vis te verminder deur inligting aan die verbruiker te verskaf wat sal lei tot die vrywillige vermindering van die risiko tot by aanvaarbare vlakke. 'n Oorsig van bestaande literatuur toon aan dat verskeie ondersoeke oor die chemiese besoedelingsvlakke in varswatervisse van Suid Afrika onderneem is. Die meeste van hierdie studies is gefokus op spesie- en weefselverskille in bioakkumulering van besoedelingstowwe sowel as die ruimtelike- en temporele variasies in besoedelingsvlakke om 'n bydrae tot die evaluering van die akwatiese omgewing se gesondheid te lewer. Die gesondheidsrisiko vir die mens, indien die vis geëet word, is selde aangespreek. Die verskillende ondersoeke volg ook nie 'n standaard metodologie soos deur die 'US EPA' voorgestel nie. Om hierdie tekortkominge aan te spreek, is die hoofdoel van hierdie skripsie om 'n generiese metodologie te ontwikkel wat gebruik kan word om ondersoeke uit te voer wat die gesondheidsrisiko's verbonde aan die eet van chemies gekontamineerde varswatervis deur ontspannings- en bestaan-vissers te bepaal. Verder sal die metodologie ook leiding gee aan ondersoeke wat die besoedelingsvlakke van chemiese kontaminate in varswatervis as deel van omgewings-gesondheidbepalings wil uitvoer.

Die basis van die metodologie is opvanggebiedsinligting (menslike aktiwiteite wat tot besoedeling kan lei), sosio-demografiese inligting van die verbruikers van varswatervisse in die opvanggebied, bioakkumuleringspotensiaal en gesondheidsrisiko van chemiese besoedelingstowwe, moniteringsontwerp, risiko-bepalingsprosedures en monitering wat op verskillende vlakke uitgevoer word. Die metodologie identifiseer tien hoofstappe naamlik (i) seleksie van die omvang van die ondersoek, (ii) evaluering van die watermassa-opvanggebied, (iii) moniteringsisteem ontwerp, (iv) veldinsameling, (v) laboratorium monster prosessering en ontleding, (vi) ontleding en rapportering van resultate, (vii) risiko-bepaling, (viii) risiko-bestuur, (ix) risiko-kommunikasie en (x) hersiening van die program, wat bespreek word om sodoende leiding aan nasionale staatsdepartemente, plaaslike owerhede en programbestuurders te gee. Die basiese vereistes van elke stap word uitgelig omdat beperkte hulpbronne (finansieël,

infrastruktuur en opgeleide personeel) in Suid Afrika dit in baie gevalle onmoontlik sal maak om indiepte ondersoeke soos deur die 'US EPA' voorgestel te onderneem. Nieteenstaande hiervan sal die toepassing van die voorgestelde metodologie vergelykbare beoordelings met betrekking tot die gesondheidsrisiko's wat met die eet van varswatervis (gebaseer op risiko-beginsels) van Suid Afrikaanse sisteme moontlik maak.

Die studie van die Vaalrivierkeerwaldam en die Kliprivier dui daarop dat daar 'n potensiële gesondheidsrisiko (hoofsaaklik as gevolg van nikkel vlakke) geassosieer met die daaglikse eet van vis vanaf hierdie sisteem is. Hierdie bevinding ondersteun dus die uitgangspunt dat waardevolle inligting oor die moontlike gesondheidsrisiko verbonde aan die eet van chemies gekontamineerde varswatervisse ingewin kan word indien 'n risiko gebaseerde benadering gevolg word. Die studies identifiseer ook gebiede in die akwatiese sisteem waar die akwatiese lewe en veral visse onaanvaarbare vlakke van chemiese besoedelingstowwe as gevolg van menslike aktiwiteite het. Hierdie inligting kan dus in omgewingsbestuursprogramme gebruik word en lewer dus 'n bydrae tot die bestuur van opvanggebiede in Suid Afrika

Uit die voorafgaande is dit duidelik dat indien die voorgestelde metodologie soos in die skripsie voorgestel, gevolg en geimplimenteer word, 'n waardevolle bydrae tot die beskerming van die verbruikers van varswatervis, asook tot die beskerming van die akwatiese omgewing gemaak kan word. Die studies is dus noodsaaklik indien die uiteindelike doel van ongekontamineerde varswatervisse vir die huidige sowel as toekomstige generasies verwesenlik wil word. Aangesien die Departement van Waterwese en Bosbou die bewaarder van varswatersisteme in Suid Afrika is, moet die voorgestelde metodologie deur die Departement in samewerking met ander staatsinstansies en opvangsgebiedbestuurs-agente geimplimenteer en bestuur word.

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GLOSSARY

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.

CHAPTER 1

INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Pollution of the aquatic environment is one of the worst legacies of the twentieth century. It is well documented that modern agriculture, industrialisation and urbanisation have negatively affected environmental quality and specifically aquatic systems (Förstner & Wittmann, 1983; Hellawell, 1986; Abel, 1989; Ellis, 1989; Mason, 1991; Dallas & Day, 1993; Johnson, 1996). 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 (Tchounwou *et al.* 1996). The impacts of pollutants such as pathogenic organisms, sulphates, low pH, high conductivities, high salinities, organic enrichment, biocides and metals are a cause of increasing concern to water quality managers and the general public in South Africa (DWAF, 1986; Heath, 1999).

Effects on human health as a result of exposure to surface water contaminants can occur through contact recreation, drinking water and the consumption of contaminated food for example, fish and shell fish (US EPA, 1991). During contact recreation dermal absorption and incidental ingestion may pose a potential health risk. Drinking water poses a very high health risk; however, the risk can be reduced by effective treatment and by applying drinking water criteria. People consuming fish or shellfish are potentially at risk as these organisms have the potential to bioaccumulate harmful contaminants from the aquatic environment (US EPA, 1991; Bevelhimer, 1995). The contaminants that have been bioaccumulated by the fish or shellfish pose carcinogenic, genotoxic and non-carcinogenic health risks to consumers (Reinert *et al.* 1991; US EPA, 1991). However, it must be stressed that the consumption of fish is generally beneficial as it provides a good source of protein, vitamins, omega fatty acids and basic minerals (Anderson *et al.* 1972; US EPA, 1997; Zabik *et al.* 1995). Additional benefits of consuming fish include a decrease in cardiovascular disease, a reduction in blood pressure in individuals, reduced colon and breast cancer risks, a decrease in pain from arthritis and a decrease in asthma attacks in asthmatics (US EPA, 1997). From the preceding it is evident that the consumption of fish is beneficial to humans, but if these fish are contaminated they pose a health risk to consumers.

As a result of the potential health risk associated with the consumption of chemically contaminated non-commercially caught fish, the United States of America has been issuing fish consumption advisories and bans (US EPA, 1995a,b, 1996, 1997, 1999). Fish consumption advisories are designed to reduce the risk to fish consumers by providing information that would lead to the voluntarily restriction of fish consumption to levels that pose limited, if any risk. A fishing ban, on the other hand, involves the banning of the consumption of fish by closing water bodies for fishing and/or banning the possession of contaminated fish. Therefore, the main difference between fish consumption advisories and fish advisory bans, is that the fish consumption advisories are voluntary while the advisory bans are mandatory. Furthermore, fish

consumption advisories not only aim to minimise the health risk to the consumers of fish but also intend to minimise the negative effects of restricting consumption and fishing (US EPA, 1997). The different States of the United States of America therefore issue five major types of advisories or bans to protect the population, namely:

- No-consumption advisory for the general population (**NCGP**). – This advisory is issued when the chemical contaminant levels in the fish pose a health risk to the general public.
- No-consumption advisory for sensitive sub-populations (**NCSP**). – This advisory is issued when the chemical contaminant levels in the fish pose a health risk to sensitive sub-populations, for example, to pregnant women, nursing mothers and children.
- Restricted consumption advisory for the general population (**RCGP**). – This advisory is issued when the chemical contaminant levels in the fish is less severe and it is recommended that the general public restrict their consumption of a specific species for which the advisory is issued.
- Restricted consumption advisory for sensitive sub-populations (**RCSP**). – This advisory is issued when the chemical contaminant levels in the fish are less severe and it is recommended that sensitive sub-populations restrict their consumption of a specific species for which the advisory is issued.
- Commercial fishing bans (**CFB**). – This advisory prohibits the commercial harvest and sale of fish from a specific waterbody. Recreational use of the fish will therefore also be banned (US EPA, 1999).

Although the United States of America has issued fish contaminant advisories since the mid-1970s the various Agencies have employed different methods to estimate the risks to human health from the consumption of chemically contaminated fish. Subsequently the United States of America Environmental Protection Agency (US EPA) has developed a series of four documents to provide guidance to Agencies issuing fish consumption advisories for non-commercial fish. The four documents comprise the *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories* and address fish sampling and analysis (US EPA, 1995a), risk assessment and the calculation of fish consumption limits (US EPA, 1997), risk management (US EPA, 1996) and risk communication (US EPA, 1995b). From these documents it is evident that a fish consumption advisory programme should consist of, (i) fish sampling and analysis, therefore the collection of contaminant data (ii) risk assessment (iii) risk management and (iv) a risk communication and associated health advisory programme. However, much of the information and guidance provided in these documents has a wider application and could assist in the development of any investigation related to the assessment of contaminant levels in fish and shellfish.

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) 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

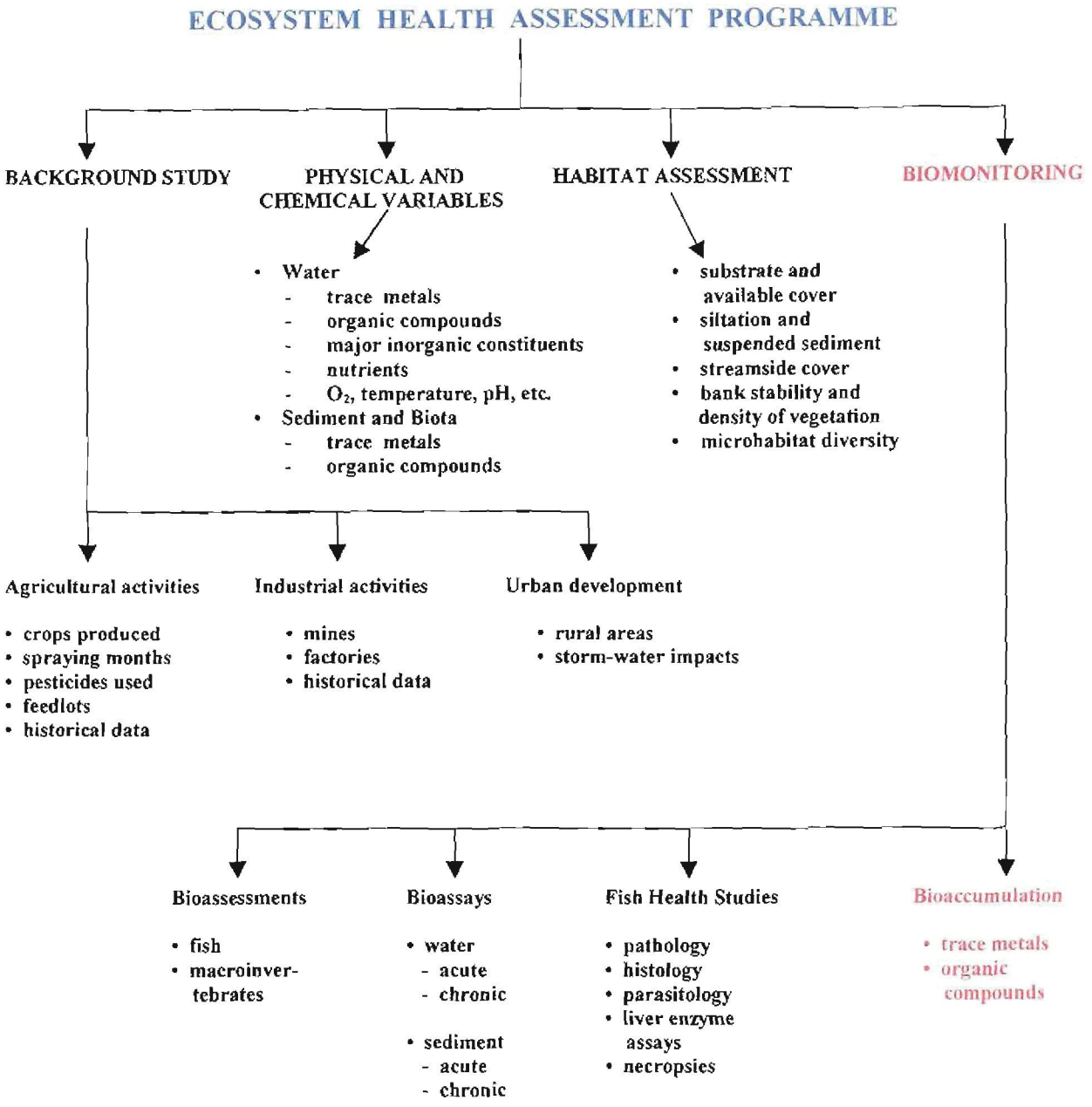

Figure 1.1: Conceptual framework of a monitoring programme to assess aquatic ecosystem health/integrity (Roux *et al.* 1993).

of the aquatic ecosystem under investigation (Figure 1.1). 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 saxitoxins (Branch & Branch, 1981; WHO, 1999).

From the preceding it is evident that possible human health risks due to the consumption of contaminated fish from South African freshwater systems have received little attention. This is an unacceptable situation since pollutants from various anthropogenic activities are polluting these systems (Heath, 1999). Furthermore, fish are captured from many of the waterbodies in South Africa by recreational and subsistence fisherman, while commercial fishing and cage culture are undertaken at selected systems. Therefore, certain sections of the South African population that consume fish may be at risk from the possible exposure to contaminants accumulated by fish captured from freshwater systems. Information regarding the possible health risk due to the consumption of fish from the freshwater systems in South Africa is therefore urgently required.

In this dissertation guidance on methods for sampling and analysing chemical contaminants in fish as well as the risk methodology is given. This would ensure that the different Governmental Agencies in the various Provinces in South Africa follow the same methodology for fish contaminant investigations or when deriving risk-based fish consumption limits. Furthermore, this would ensure 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, 1995a). This is supported by the study by Heath (1999), which was the first attempt to standardise and give some guidance on how to perform chemical (pesticides and metals) contaminant bioaccumulation monitoring programmes in South Africa (Figure1.2). However, in the study by Heath (1999) many of the elements of a fish chemical contaminant survey 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.

It is evident that in South Africa there is a need to standardise the methodology for conducting chemical contaminant surveys using fish and to use this data to protect the health of consumers of freshwater fish. The general objective of this dissertation is to develop a generic methodology 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. It must, however, be stressed that developing and implementing methodologies to manage and reduce the human health risk associated with the consumption of freshwater fish will also benefit the aquatic ecosystem at large. The ecosystem will benefit as the ultimate goal of the management strategy would be to protect the freshwater aquatic environment and to put remedial actions in place that would ensure that the fish populations of the system are fit for present and future human consumption. The concept of sustainable development - 'development that delivers basic environmental, social and economic services to all without threatening the viability of the natural, built and social systems upon which these services depend' (ICLEI, 1995; Walmsley & Pretorius, 1996) would be underpinned as the sustainable use of a renewable resource, namely fish, would be propagated. Furthermore, some of the principles and intentions of two important Acts - namely the National Water Act (36/1998) and the National Environmental Management Act (107/1998) - would also be subscribed to as both the human

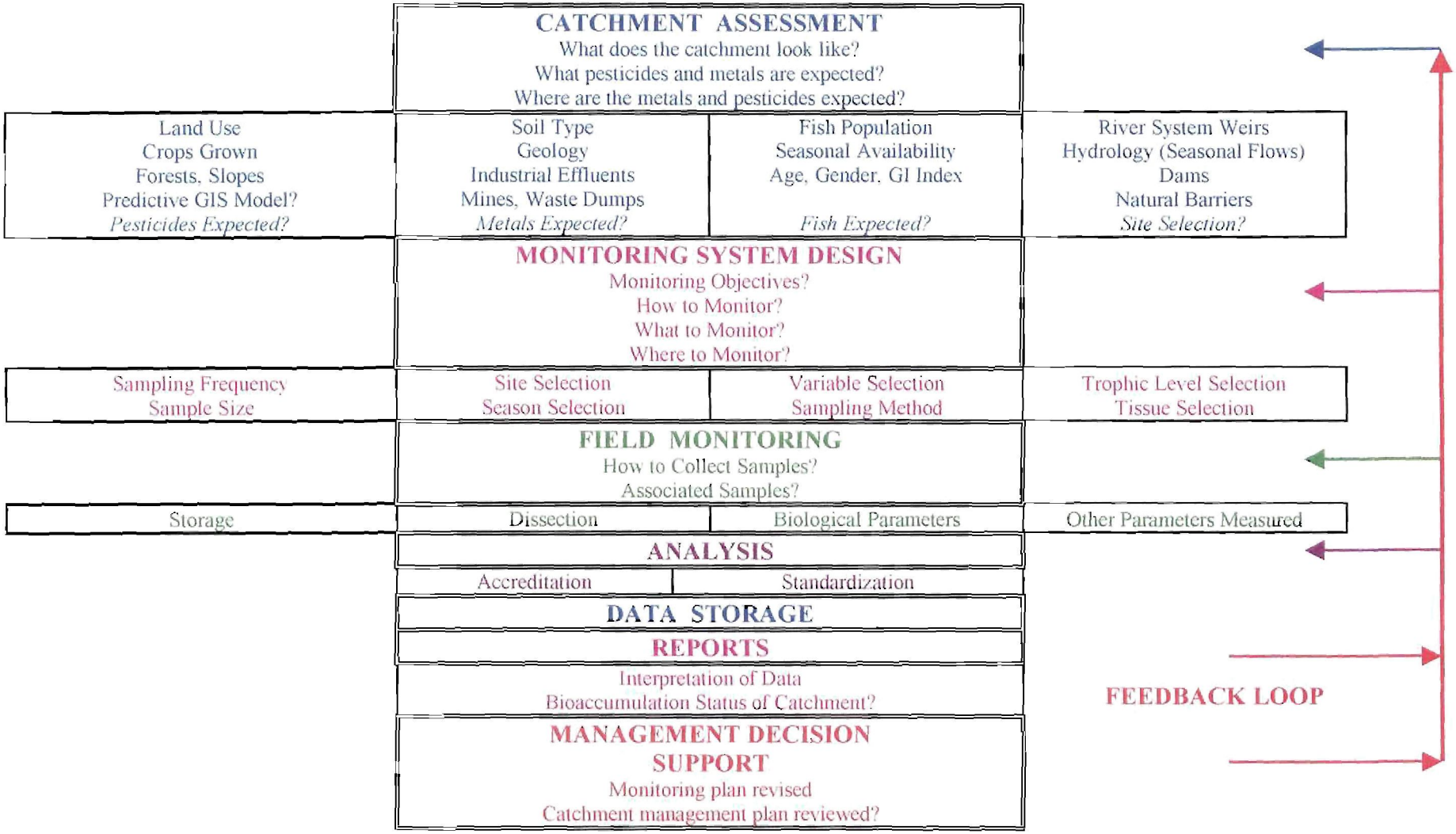

FIGURE 1.2: Bioaccumulation protocol and feedback loop proposed by Heath (1999).

population and the natural environment will benefit by following and implementing the proposed methodology.

As it is envisaged that developing a generic methodology is a complex procedure consisting of various aspects, the specific objectives of this dissertation are to:

- *Investigate the strategies and elements of freshwater fish chemical contaminant monitoring programmes.*
- *Investigate the steps of risk assessment procedures when undertaking fish contaminant assessments in relation to the consumption of contaminated fish by recreational and subsistence fishermen.*
- *Apply some of the stated methodologies to assess the possible health risks associated with the consumption of fish captured from the Vaal River Barrage Reservoir and the Klip River.*
- *Develop a generic methodology for undertaking fish chemical contaminant surveys for the consumption of freshwater fish.*

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CHAPTER 2

STRATEGIES AND ELEMENTS OF A CHEMICAL CONTAMINANT PROGRAMME FOR THE CONSUMPTION OF FRESHWATER FISH

CHAPTER 2

STRATEGIES AND ELEMENTS OF A CHEMICAL CONTAMINANT MONITORING PROGRAMME FOR THE CONSUMPTION OF FRESHWATER FISH

2.1 INTRODUCTION

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 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 programmes 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 (1995) 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. This section of the study gives guidance on undertaking chemical contaminant surveys using freshwater fish. The elements and details discussed in the sections that follow are mainly based on the guidelines given by the US EPA (1995), the protocol of Heath (1999), other South African studies on freshwater fish (Bezuidenhout *et al.* (1990); du Preez & Steyn, (1992); de Wet *et al.* (1994); Grobler *et al.* (1994); Seymore (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); Janse van Rensburg (1997); Robinson & Avenant-Oldewage (1997); Marx & Avenant-Oldewage (1998); Heath, (1999); Heath & Claassen (1999) and Nussey *et al.* (1999, 2000) and experience gained during the field surveys (see Chapter 4).

In this section of the present study the following will be addressed:

- *The monitoring strategies.*
- *Elements of the monitoring strategy, namely: (i) objectives; (ii) selection of sampling sites; (iii) selection of analytes and analytes screening concentrations; (iv) selection of species; (v) sampling sites; (vi) number of samples; (vii) sampling times and sampling frequency; (viii) sample collection; (ix) sample handling; (x) sample processing; (xi) distribution of sample; (xii) sample analysis; and (xiii) data analysis and reporting of results.*

It must be noted that this section was compiled as a more detailed report by Du Preez *et al.* (2000).

2.2 MONITORING STRATEGIES

To optimise resources and to be more cost-effective a monitoring strategy consisting of three levels was applied in a hierarchical manner:

- **Level 1:** *Screening surveys* – A national survey of water-bodies where freshwater fish are captured for commercial, subsistence or recreational purposes. Fish are therefore selected from sites 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.

2.3 ELEMENTS OF THE THREE LEVEL MONITORING SURVEYS

2.3.1 Objectives

The main objective of Level 1 surveys should be to 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.

2.3.2 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. 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.

2.3.3 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. The process of analyte selection is tedious and in many instances resource limited. **For the South African situation the following procedures are therefore recommended:**

- Selected the analytes as proposed by the US EPA (1995) as test analytes (Table 2.1) 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.

TABLE 2.1: Recommended analytes, screening concentrations and risk values for the selected analytes (adapted from the US EPA, 1995, 1997).

Selected analyte	Non-carcinogens	Carcinogens	SC[A] (µg/ℓ)	
	RFD[B] (mg/kg/day)	SF[B] (mg/kg/day)$^{-1}$	Non-carcinogens	Carcinogens (RL=10^{-5})
Metals				
Arsenic (inorganic)[C]	3×10^{-4}	1.5	3	-
Cadmium	1×10^{-3}	NA	10	-
Mercury[E]				
Developmental	1×10^{-4} [F]	NA	1	-
Chronic systemic	1×10^{-4} [F]	NA	1[F]	-
Selenium[G]	5×10^{-3}	NA	50	-
Tributyltin	3×10^{-5}	NA	0.3	-
Organochlorine Pesticides				
Total chlordane (sum of cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane)[H]	6×10^{-5}	1.3	0.6	0.08
Total DDT (sum of 4,4[1]- and 2,4[1]-isomers of DDT, DDE, and DDD)[I]	5×10^{-4}	0.34	5	0.3
Dicofol	1.2×10^{-3} [J]	0.34	10	-
Dieldrin	5×10^{-5}	16	0.6	7×10^{-3}
Endosulfan (I and II)	6×10^{-3} [J]	NA	60	-
Endrin	3×10^{-4}	NA	3	-
Heptachlor epoxide	1.3×10^{-5}	9.1	0.1	0.01
Hexachlorobenzene	8×10^{-4}	1.6	9	0.07
Lindane (ϒ-hexachloro-cyclohexane; ϒ-HCH)	3×10^{-4}	1.3 [K]	3	0.08
Mirex	2×10^{-4}	1.8[L]	2	-
Toxaphene	3.6×10^{-4} [JM]	1.1	3	0.1
Organophosphate Pesticides				
Chlorpyrifos	3×10^{-3}	NA	30	-
Diazinon	9×10^{-5} [J]	NA	0.9	-
Disulfoton	4×10^{-5}	NA	0.5	-
Ethion	5×10^{-4}	NA	5	-
Terbufos	1.3×10^{-4} [J]	NA	1	-
Chlorophenoxy Herbicides				
Oxyfluorfen	3×10^{-3}	1.28×10^{-1}	30	0.8
PAHs	NA	7.3 [N]	-	0.01
PCBs				
Total PCBs (sum of Aroclors)				
Developmental	2×10^{-5} [O]	-	-	-
Chronic systemic	2×10^{-5} [O]	2.0	0.2	0.01
Dioxins/furans[P]	NA	1.56×10^{5}	-	7×10^{-7}

TABLE 2.1: (Continued).

NA	=	Not available in EPA's Integrated Risk Information System (IRIS, 1992,1997).
PAH	=	Polycyclic aromatic hydrocarbon.
PCB	=	Polychlorinated biphenyl.
RFD	=	Oral reference dose (mg/kg/day).
RL	=	Risk level (dimensionless).
SC	=	Screening concentration.
SF	=	Oral slope factor $(mg/kg/day)^{-1}$.

A Except for mercury, screening concentrations (for Level 1 surveys) are selected analyte concentrations in fish tissue that equal exposure levels at either the RFD for noncarcinogens or the SF and an $RL=10^{-5}$ for carcinogens, given average consumption rates (CRs) and body mass (BMs) of 6.5 g/day and 70 kg, respectively, for the general adult population.

B Unless otherwise noted, values listed are the most current oral RFDs and SFs from IRIS (1995, 1997).

C Total inorganic arsenic should be determined for comparison with the recommended SC.

D From US EPA (1997).

E Because most mercury in fish and shellfish tissue is present as methylmercury and because of the relatively high cost of analyzing for methylmercury, it is recommended that total mercury be analyzed and the conservative assumption be made that all mercury is present as methylmercury.

F The US EPA has recently re-evaluated the RFD for methylmercury, primarily because of concern about evidence that the fetus is at increased risk of adverse neurological effects from exposure to methylmercury. An oral RFD of 1×10^{-4} mg/kg/day based on developmental neurological effects in human infants was included. This oral RFD of 1×10^{-4} mg/kg/day is considered protective for chronic systematic effects of methylmercury among the general adult population, women of reproductive age, and children.

G The RFD for selenium is the IRIS (1997) value for selenious acid. The evidence of carcinogenicity for various selenium compounds in animal and mutagenicity studies is conflicting and difficult to interpret.

H The RFD and SF values listed are derived from studies using technical-grade chlordane (purity 95%) or a 90:10 mixture of chlordane:heptachlor or analytical-grade chlordane. No RFD or SF values are given in IRIS (1992, 1997) for the cis- and trans-chlordane isomers or the major chlordane metabolite, oxychlordane, or for the chlordane impurities cis- and trans- nonachlor. It is recommended that the total concentration of cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane be determined for comparison with the recommended screening concentration (SC).

I The RFD value listed is for DDT. The SF value is for DDT or DDE; the SF value for DDD is 0.24. The use of SF = 0.34 for any combination of DDT, DDE, DDD, and dicofol is recommended. It is recommended that the total concentration of the 2,4' – and 4,4'-isomers of DDT and its metabolites, DDE and DDD, be determined for comparison with the recommended SC.

J The RFD value listed is from the Office of Pesticide Program's Reference Dose Tracking Report, US EPA.

K The SF value listed for lindane was calculated from the water quality criteria (0.063 $\mu g/\ell$).

L The National Study of Chemical Residues in Fish used a value of SF = 1.8 for mirex (HEAST, 1989).

M The RFD value has been agreed upon by the Office of Pesticide Programs and the Office of Water of the United States of America.

N The SF value listed is for benzo[a]pyrene.

O The RFD for PCBs is based on the chronic toxicity of Aroclor 1254.

P The SF value listed is for 2,3,7,8-tetrachlorodibenzo-p-dioxin (US EPA, 1995, 1997).

It is important to stress that the list of analytes can be refined by:

- 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) and Heath & Claassen (1999), 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, 1995). An example of the contaminants from such an investigation is presented in Table 2.1.
- 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 2.1).
- 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, 1995). 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 (1991) and Streit (1998).

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, 1995). The US EPA (1995) 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, 1995).

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

$$\mathbf{SC_c = [(RL/SF) \times BM]/CR} \qquad \mathbf{(2.1)}$$

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:

$$\mathbf{SCn = (RFD \times BM) / CR} \qquad \mathbf{(2.2)}$$

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 2.2) 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).
- 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).

To obtain calculated (oral) RFD and/or SF"s for chemical contaminants (including all the selected analytes listed in Table 2.1) the 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, 1995).

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, 1995). To address uncertainty (Table 2.2) 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 2.1). 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).

TABLE 2.2: 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.

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

$$SC_c = (RL \times BM) / SF \times \{[WI/(BCF \times FM \times LR)] + CR\} \quad (2.3)$$

where:

- SCc = Screening concentration for a carcinogen (mg/kg).
  RL = Maximum acceptable risk level (dimensionless). This is the assigned level o f 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).

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

$$SCn = [(RFD \times BM) - (DT + IN) \times BM] / [WI / (BCF \times FM \times LR)] + CR \quad (2.4)$$

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 2.2.
- 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 (1995) is used. The screening concentrations as listed in Table 2.4 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 2.1. 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 2.1 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™ 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™ 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 obtained chemical contaminant concentrations are directly applied in the Risk *Assistant™ software package.

2.3.4 Species selection

In South Africa various freshwater species have been used to investigate the levels of selected biocides and metals. 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, 1995; 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, 1995).

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, 1995).

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, the fish species listed in Table 2.3 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 finding of the US EPA (1995), Heath (1999) and the data summarised in Table 2.3 the following is recommended:

- **Level 1:** *Screening surveys* – At least one bottom feeder or one predator species selected from the species in Table 2.3. Preferably include one bottom feeder and one predator species.

TABLE 2.3: Freshwater fish species that are recommended for consideration for chemical contaminant investigations in South Africa.

FAMILY NAME	SCIENTIFIC NAME	COMMON NAME	FEEDING HABITS
CYPRINIDAE	*Barbus aeneus*	Small mouth yellow fish	Bottom feeder, omnivorous
	Barbus andrewi [1]	White fish	Bottom feeder, invertebrates and algae
	Barbus natalensis [2]	Scaly	Omnivorous, algae, invertebrates, detritus
	Barbus 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

- **Level 2:** *Intensive surveys, Phase I* – 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.

2.3.5 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 I* – 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.

2.3.6 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, 1995). 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 in Section 2.3.11. For scaleless fish species, for example the African sharptooth catfish (*Clarias gariepinus)*, the skin should be removed (US EPA, 1995). 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. 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 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, 1995).

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.
- 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 (see Section 2.3.8).
- 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 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 2.7). 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.

2.3.7 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_0 and the alternative hypothesis, H_A, where:

H_0 = 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 (1995) 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_0 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.
- 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, 1995). 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, 1995).

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) and the present study (see Chapter 4) 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.

2.3.8 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 (1995) 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 (1995) 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.

2.3.9 Sample 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 2.4). **It is recommended Table 2.4 can be used as a guide for fish sampling methods for South African water-bodies.**

2.3.10 Sample handling

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 (1993) 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.

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, 1995).

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 2.1. Other external and internal features of a typical bone fish are shown in Figure 2.1 and Figure 2.2.

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).

TABLE 2.4: Fish sampling methods that can be used in South Africa (adapted from US EPA, 1995).

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.

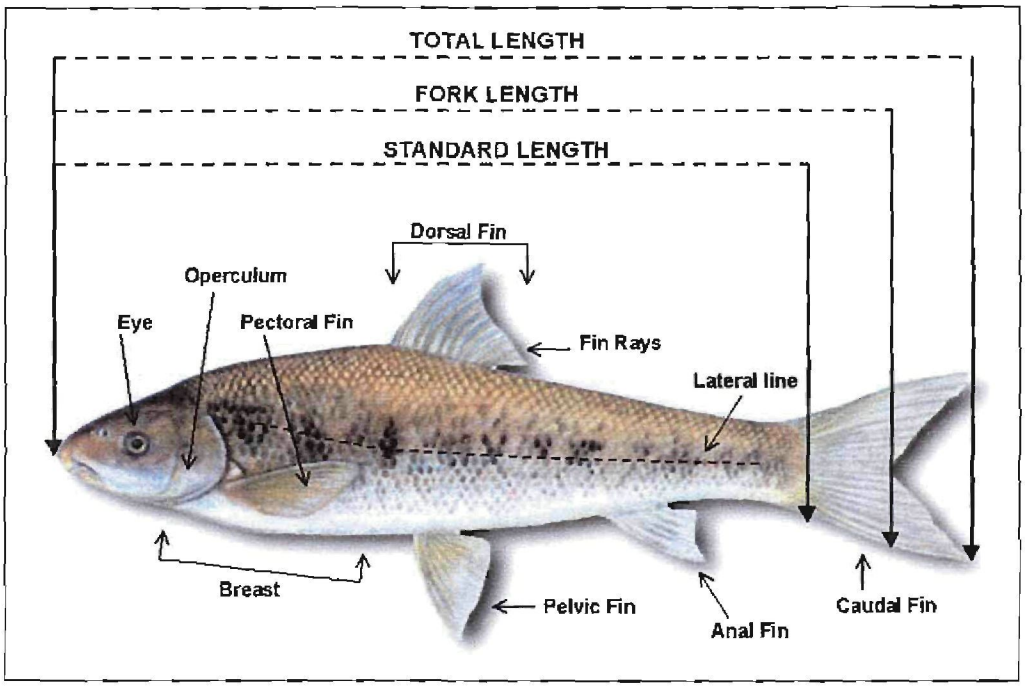

FIGURE 2.1: External features and size measurements of a freshwater bone fish (adapted from Skelton, 1993).

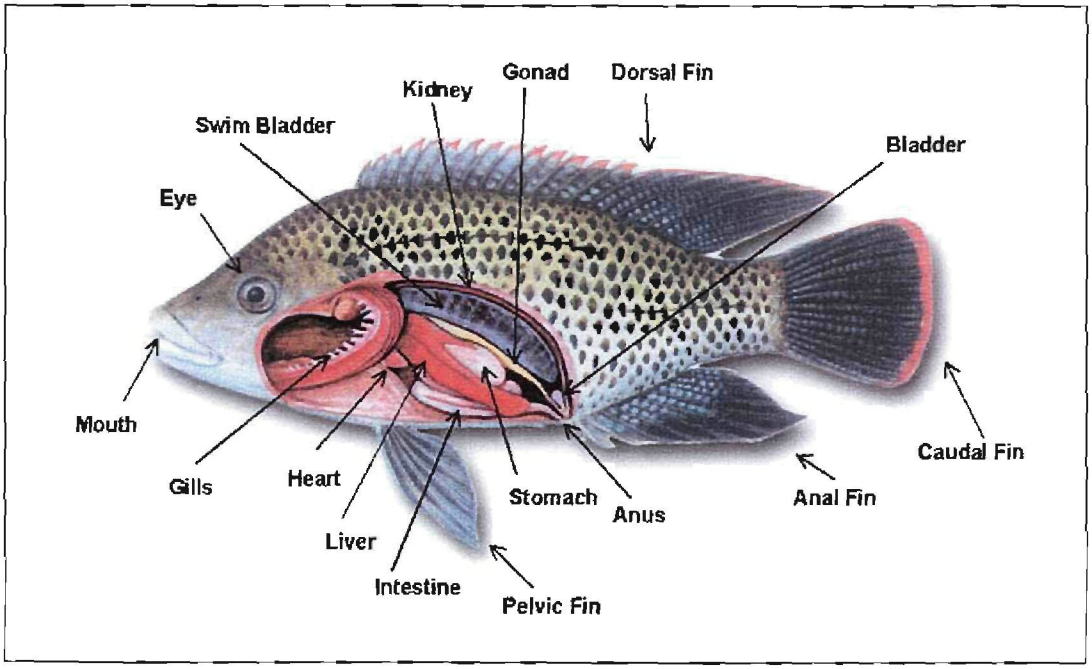

FIGURE 2.2: External features and internal organs of a freshwater bone fish (adapted from Skelton, 1993).

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. 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. 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 2.2) 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. It is therefore recommended that the health of the selected specie is evaluated according to a Fish Health Assessment Index (FHAI) consisting of the following variables:

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

The description of these variables and the associated sources are summarised in Table 2.5. 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.

TABLE 2.5: Fish Health Assessment Index (FHAI) variables and assigned values. 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 color	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

Sample packaging and preservation

Each fish should be individually wrapped in extra heavy aluminium foil and placed in a waterproof plastic bag (Table 2.6). 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, 1995).

Depending on the transport time the samples can be kept on wet ice packets or frozen on dry ice (Table 2.6). 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 2.7.

TABLE 2.6: Recommended preservation of fish samples from time of collection to delivery at the laboratory (adapted from the US EPA, 1995).

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

Table 2.7: 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, 1995).

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 ≤ -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 ≤ -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 ≤ -20°C.	1 year

Table 2.7: (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 ≤ -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 ≤ -20°C.	1 year

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, 1995; du Preez, 1999; du Preez *et al.* 2000):

- Field sample request form
- Field sampling record form
- Fish Health Assessment index Form
- Fish sample Identification label
- Chain of custody label
- Chain of custody record form

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. Examples of these forms can be found in the publications of the US EPA (1995) and du Preez *et al.* (2000). Furthermore, document control requirements as described in the above mention international standards should be implemented.

2.3.11 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, 1995).

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, 1995). 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, 1995). The suggested sample processing equipment, container materials and holding time for fish are summarised in Table 2.7.

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, 1995). 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 envelopes or plastic bags, 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).

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 2.2). The gender of the fish and stage of reproduction can then be determined by using the classification system of gonad development (Table 2.8) 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. The fish health assessment as described in Section 2.3.10 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 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, 1995). Puncturing of internal organs must be avoided, as the material released from the internal organs will contaminate the fillets. After removing of the fillets they are weighed after which they are processed further or stored (Table 2.7).

Preparation of individual and composite homogenates

The fillets from individual fish must 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, 1995). Thereafter the individual homogenates are either processed further to prepare composite homogenates or stored separately as indicated in Table 2.7.

TABLE 2.8: 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 $\frac{1}{3} - \frac{1}{2}$ 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.

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 size fillet of combined fillet). 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 2.7. 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, 1995). The various steps of the preparation of fish fillet homogenates are summarised in Figure 2.3.

2.3.12 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 2.22) and the transfer of the aliquots to a specific laboratory (Table 2.23) must be kept.

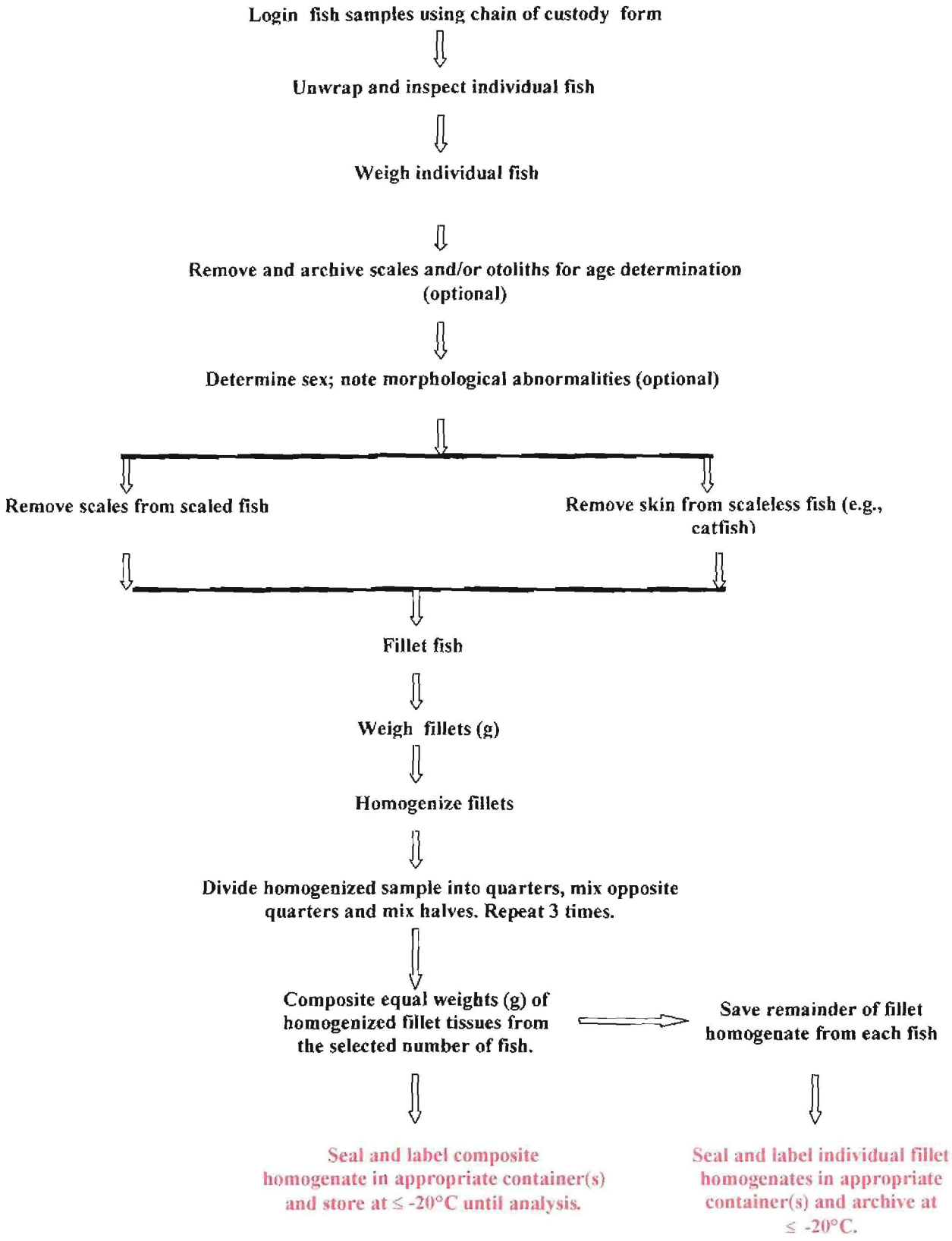

FIGURE 2.3: Summary of the steps in the laboratory preparation of fish fillet composite homogenate samples (adapted from the US EPA, 1995).

2.3.13 Sample analysis

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). 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, 1995).

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 (1995) 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, 1995).

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, 1995). 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, 1995).

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 (1995) 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.**

2.3.14 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, 1995).

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, 1995).

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 2.4 (US EPA, 1995). 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, 1995).
- 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.

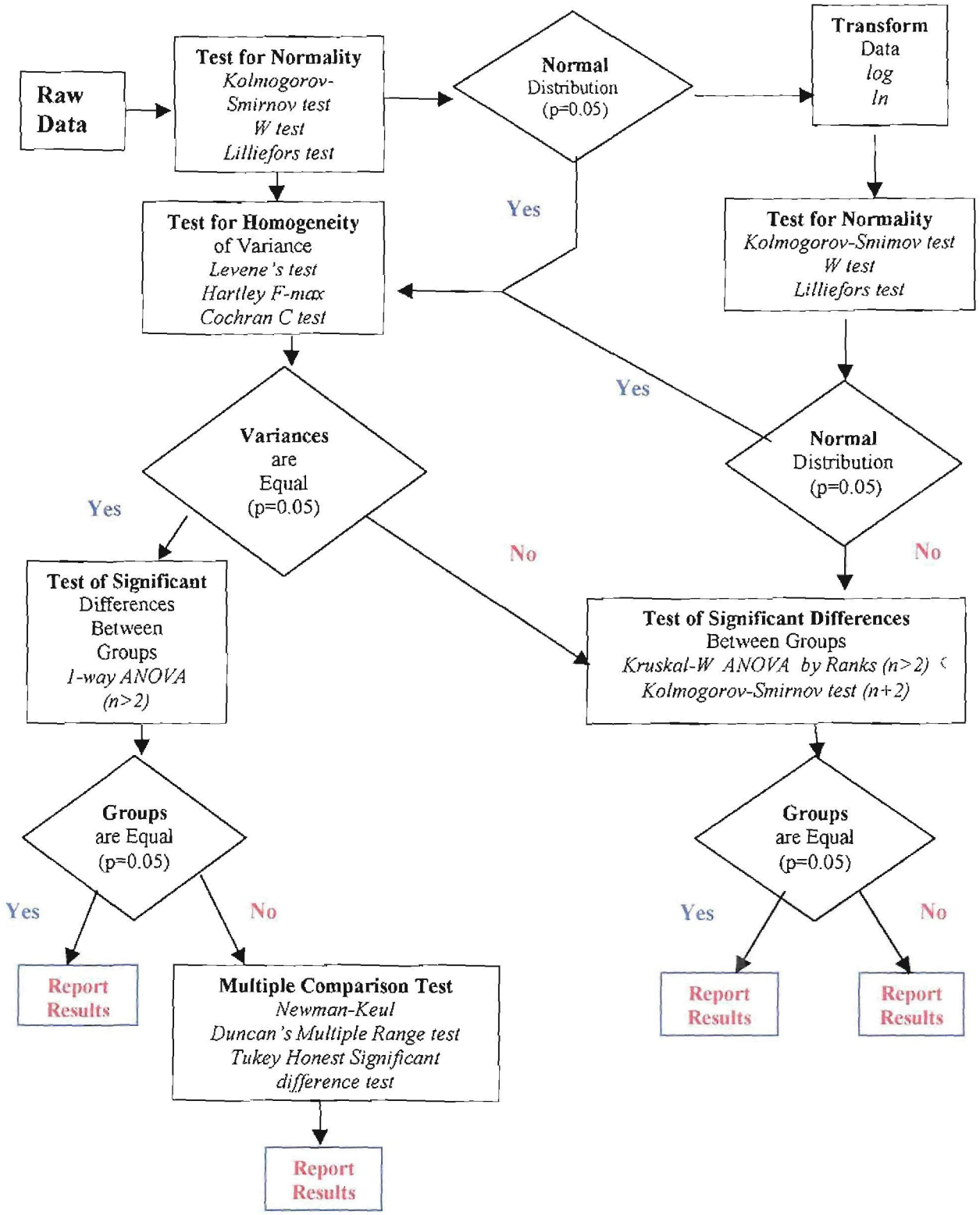

FIGURE 2.4: Statistical approach for testing of statistically significant differences between fish contaminant survey data sets (adapted from the US EPA, 1995).

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 digestates).
- 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).

2.4 CONCLUSIONS AND RECOMMENDATIONS

The United States of America Environmental Protection Agency has developed and implemented well-defined strategies and procedures for chemical contaminate monitoring of freshwater fish species used by recreational fisherman as food. These strategies and procedures are well documented and can be used as guidance documents to develop specific programmes. The main shortcoming of these strategies and procedures is that they would require resources (infrastructure, human and financial) that would not always be available to developing countries like South Africa. These strategies and procedures should therefore be used to guide the development of strategies, procedures and programmes that would fulfill local requirements considering resource constraints.

Surveys designed to investigate the chemical contaminant concentrations in fish tissue should be well planned to ensure that the objectives are clearly defined, achievable and eventually reached. The three monitoring levels proposed should assist in this process. The selection of analytes is important and should be based on a knowledge of anthropogenic activities in the catchment area, bioaccumulation potential of the analyte, possible health risk to consumers, recommended analytes by other countries and information from previous fish consumption advisories or fishing bans. When undertaking these surveys the fish tissue collection and analyte analysis procedures described in this chapter should be followed. However, in some cases certain activities are not feasible or not applicable to the specific investigation. The project team should therefore, evaluate the suggested procedures and adapt them if deemed necessary to achieve the specific programme objectives. Nevertheless, the tissue collection and analyte analysis procedures must ensure accuracy and reliability of the results. All the databases for fish chemical contaminant surveys should be structured in such a manner that information and/or data can be entered in the suggested datafields. The Department of Water Affairs and Forestry must develop and maintain the National Chemical Contaminant Database for the central storage of chemical contaminant data. This database will store all the data of the fish chemical contaminant surveys for the assessment of possible human health risks as well as any data relating to chemical contamination obtained during the general catchment monitoring programmes or specific special investigations.

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ISRS **see** INTERNATIONAL SAFETY RATING SYSTEM.

ISO/IEC **see** INTERNATIONAL STANDARDS ORGANISATION

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CHAPTER 3

RISK ASSESSMENT OF FISH CHEMICAL CONTAMINANT DATA

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3.1 INTRODUCTION

One of the main pathways of human exposure to chemical contaminants from the aquatic environment is through the consumption of contaminated food, for example fish or shellfish that have the potential to bioaccumulate harmful contaminants from a polluted aquatic system (US EPA, 1991a; Bevelhimer, 1995). People consuming the chemical contaminated food are potentially at risk as they are exposed to pollutants that may cause carcinogenic (non-threshold effects), genotoxic (non-threshold effects), or non-carcinogenic (threshold effects) health effects. Non-threshold pollutants generally have no level of exposure that does not pose a small, finite probability of generating a carcinogenic response, while a threshold pollutant has an identifiable exposure threshold below which effects are not observed (US EPA, 1991a; Reinert *et al.* 1991).

A classic example of the risks to human health by eating contaminated food is the well-known case of the methyl mercury poisoning of people from the village of Minimata Bay, Japan. Eating methyl mercury contaminated fish from Minimata Bay was linked to several deaths, cases of brain damage and newborn babies with neurological problems (Smith & Smith, 1975). It is therefore evident that the consumption of chemically contaminated fish may pose an unacceptable human health risk to consumers. The health risks result from the actual or potential exposure of the consumer to a hazard contaminant and are therefore a combination of the defined contaminant hazard and the magnitude of the effects it elicits (Risk *Assistant^{TM}, 1995; HMSO, 1998). The contaminant hazard arises from the way the contaminant and the exposed individual interact. It is usually described in terms of the adverse effect or effects that the contaminant produces in humans as defined by the relationship between the amount of contaminant and the potential severity of its adverse effects or in relation to the frequency of occurrence in the human population (Risk *Assistant^{TM}, 1995).

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 3.1). 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 be perform human

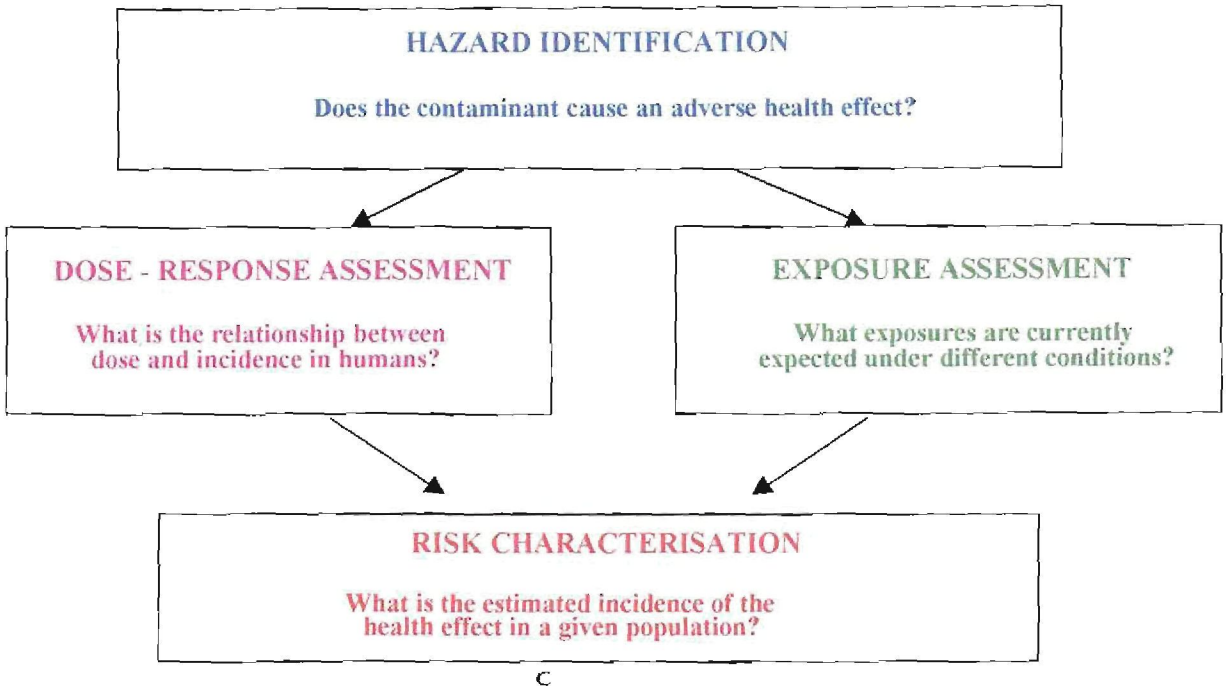

FIGURE 3.1: Steps of the risk assessment process (adapted from NAS, 1983).

health risk assessment in relation to the consumption of contaminated freshwater fish.

The objectives of this section of the study are to briefly describe (i) the various steps of risk assessment in relation to the consumption of contaminated fish as recommended by the US EPA (1997) and (ii) the application of the Risk *Assistant™ software package.

3.2 STEPS OF RISK ASSESSMENT

3.2.1 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 (see Chapter 2: Section 2.3.3) 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; 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.

These aspects have been discussed in more detail in Chapter 2 (see Section 2.3.3) of this dissertation.

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 it is advisable to perform more detailed hazard identification 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 due to the variability in persistence and bioaccumulation potential in complex aquatic systems that have not been evaluated. The persistence and bioaccumulation potential of new chemicals may not have been fully evaluated.
- 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.

3.2.2 Dose-response assessment

In this step 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 (see Chapter 2: Section 2.3.3) have been derived (US EPA 1995a, 1997) and are summarised in Table 2.2 (see Chapter 2: Section 2.3.3). 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 (see Chapter 2: Section 2.3.3) 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 (Table 2.1: Chapter 2) for some of the selected analytes is provided in Chapter 2 (see Section 2.3.3) of this dissertation.

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 (see Chapter 2: Section 2.3.) 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.
- The assumption that there is a threshold for most non-carcinogens and no threshold for carcinogens.
- Uncertainty as a result of differences between animal studies and real human populations. Furthermore, human data is usually derived from occupationally exposed people.
- 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).

3.2.3 Exposure assessment

This step 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.

When performing exposure assessment for the consumption of freshwater fish, information and data on chemical residues in the fish and human consumption patterns are used to identify and describe potentially exposed populations. In the section that follows the main aspects that are usually addressed are briefly discussed.

Chemical contaminant (analyte) concentrations in fish

Various factors related to the fish species (specific species, size class, type of tissue, trophic status, physiological condition etc.), the contaminant (bioaccumulation potential, persistence, biochemical fate, etc.) and geographical distribution of contaminants will influence the type and concentrations of contaminants detected in fish tissue (US EPA, 1997; Streit, 1998; du Preez, 1999). Fish contaminant surveys must therefore be designed to address these variations in order to achieve the objective of protecting the consumer's health. The various aspects of undertaking fish contaminant surveys are discussed in Chapter 2 of this dissertation. Reference to publications related to contaminant surveys in South Africa is made in Chapter 2 (see Sections 2.1 and 2.3.3).

Geographical distribution of contaminated freshwater fish

This information is useful in performing risk characterisation during population exposure assessment and in determining the need for further action (US EPA, 1997). Information on where contaminated fish have been found and the source of potential contamination (from catchment situation analysis, pollution incidents etc.) is usually obtained during Level 3 surveys for evaluation. Some information (although limited) relating to the geographical distribution of contaminants in South Africa freshwater fish can be found in the publications listed in Chapter 2 (see Sections 2.1 and 2.3.3) of this dissertation.

Socio-demographic information and data on fish consumption patterns

To perform individual or population exposure assessments it is essential to obtain socio-demographic information (age, sex, body mass, etc.) on the local population. Local 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. In many cases this information is not available for the general population at large or specific sub-populations. General derived values can thus be used as indicated in Table 3.1. However, where possible, information on specific sub-populations must be obtained.

To gather this information specific fish consumption surveys are undertaken using methods such as telephone surveys, mail surveys, personal interviews, daily record-keeping and creel census (US EPA, 1992). 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 3.2 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 3.2 can be adapted according to the survey objectives, the resource limitations and the local conditions. Furthermore, the general derived values (Table 3.1) 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.

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:

$$\mathbf{E_m = (C_m \times CR) / BM} \qquad \mathbf{(3.1)}$$

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).

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:

$$\mathbf{CR_{lim} = (ALR \times BM)/ (SF \times C_m)} \qquad \mathbf{(3.2)}$$

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:

$$\mathbf{CR_{mm} = (CR_{lim} \times TP_{ap})/ MS} \qquad \mathbf{(3.3)}$$

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:

$$\mathbf{CR_{lim} = (RFD \times BM)/ C_m} \qquad \mathbf{(3.4)}$$

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).

TABLE 3.1: 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} (unitless) 10^{-5} (unitless) 10^{-6} (unitless)
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)
Measured contaminant concentration in edible fish and shellfish tissue (C_m)[c]	mg/kg Varied with local conditions for each chemical contaminant, for each species, and for each size (age) class within a species
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.

TABLE 3.2: 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.
- Education level attained.
- Number of household members.
- Race/ethnic group, sex, age, height and mass of the fisherman 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 bottomfish, 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).

Examples of the values of the parameters for the above equations used by the US EPA (1997) to calculate risk-based consumption limits are summarised in Table 2.1, Table 2.2 (see Chapter 2: Section 2.3) and Table 3.1. 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:

$$\mathbf{E_{mj} = \Sigma(C_{mj} \times CR_j \times P_j) / BM} \qquad \mathbf{(3.5)}$$

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:

$$\mathbf{CR_{tm} = \sum_{j+1}^{n}(CR_{mj} \times P_j)} \qquad \mathbf{(3.6)}$$

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:

$$\mathbf{CR_{lim} = (ALR \times BM) / \sum_{j+1}^{n}(CR_{mj} \times P_j) \times SF} \qquad \mathbf{(3.7)}$$

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:

$$\mathbf{CR_j = CR_{lim} \times P_j} \qquad \mathbf{(3.8)}$$

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 3.3 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:

$$\mathbf{CR_{lim} = (RFD \times BM) / \sum_{j+1}^{n} (CR_{mj} \times P_j)} \qquad \mathbf{(3.9)}$$

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:

$$\mathbf{CR_{lim} = (ALR \times BM) / \sum_{m+1}^{x} [\sum_{j+1}^{n} (CR_{mj} \times P_j)] \times SF_m} \qquad \mathbf{(3.10)}$$

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 3.10 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:

$$\mathbf{CR_{lim} = (ALR \times BM) / \sum_{m+1}^{x} CR_m \times SF_m} \qquad \mathbf{(3.11)}$$

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 3.3.

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:

$$\mathbf{CR_{lim} = \sum_{m+1}^{x} [RFD_m / \sum_{j+1}^{n} (CR_{mj} \times P_j)] \times BM} \qquad \mathbf{(3.12)}$$

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 3.12 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:

$$\mathbf{CR_{lim} = \sum_{m+1}^{x} [RFD_m / (CR_m)] \times BM} \qquad \mathbf{(3.13)}$$

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 3.1 to 3.13, risk values (see for example Table 2.1; Chapter 2: Section 2.3.3) and selected input values (Table 3.1). Examples of the tables that can be compiled are presented in Table 3.3 and Table 3.4. This approach was used by the US EPA (1997) to derived risk-based consumption limits for each of the selected analytes described in Table 2.1 (see Chapter 2: Section 2.3.3). **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 under investigation must be obtained. The average and maximum residence time in the area where exposure is likely to occur.
- 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.
- 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**:

$$\mathbf{E_T = [(C_m \times CR) / BM] \times E_A \times E_W \times E_F \times E_O} \qquad \mathbf{(3.14)}$$

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 3.3: 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 3.4: 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.

More detail on the methodology for the calculations of total exposure can be found in the US EPA publications (US EPA, 1991; 1997). Population exposure assessments usually include individual consumers at the central tendency and high-end portion of the exposure distribution (Figure 3.2). 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 3.2). 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 ($\geq$ 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).

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 (see Chapter 2: Sections 2.3.10 to 2.3.13).
- 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 if exposure limits consider only one fish species when the consumer population includes more than one species in their diet.
- Uncertainty in exposure assessment when consumers are exposed to other contaminant sources (from water, from air, food other than fish, soil, etc.).

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.**

3.2.4 Risk characterisation

In this final step in the risk assessment process all the information about the other three steps (hazard identification, dose-response assessment, exposure assessment) is used to characterise 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

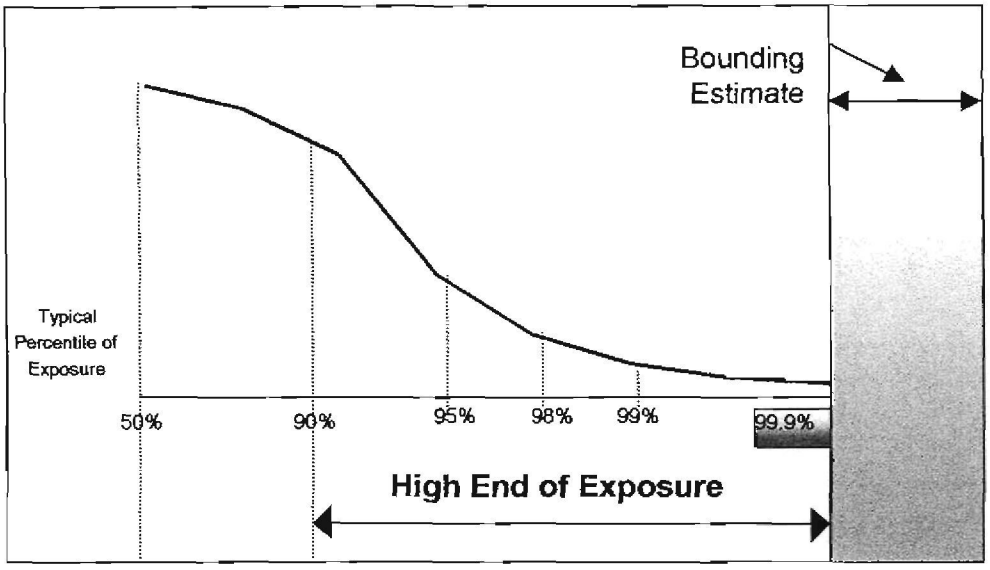

FIGURE 3.2: Schematic presentation of exposure categories in the upper half of a normal distribution (adapted from the US EPA, 1997).

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:

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

- 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:

HQ = ADD/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.

3.2.5 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 3.5)
- Risk estimates summary table (Table 3.6)
- Risk characterisation summary table (Table 3.7)
- Risk summary table for a specific water-body (Table 3.8)
- Risk summary table for a catchment or geographical area (Table 3.9)

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

3.3 THE APPLICATION OF THE RISK*ASSISTANT™ SOFTWARE PACKAGE IN RISK ASSESSMENT

The Risk*Assistant™ 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™ (Risk*Assistant™, 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.

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

TABLE 3.5: 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:

Contaminant (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 3.6: 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 3.7: 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 3.8: 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 3.9: 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:		

analysis to test the impact of different assumptions on exposure health risks. In South Africa, Claassen (1996) and Heath (1999) used the Risk*Assistant™ software package to assess the possible health risk associated with the consumption of fish from the Crocodile, Olifants, Levuvhu, Sabie and Berg rivers. These studies showed that this programme can be effectively applied to South African conditions and should be more widely 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.

3.4 CONCLUSIONS

Pollution of the aquatic environment as a result of human activities may contaminate a specific water-body to such an extent that that it poses a risk to human health. For example, fish may bioaccumulate chemical contaminants that may pose a health risk to consumers. It is therefore necessary to provide consumers with the necessary information in order to limit or prevent health risks due to the consumption of fish. This is especially true for recreational and subsistence fisherman who may be at risk from eating fish that they have been captured from contaminated water-bodies.

The health risks due to the consumption of contaminated fish are predicted during the risk assessment process by evaluating the inherent ability of a chemical to cause adverse effects

qualitatively and quantitatively at different concentrations of exposure to and the amount of exposure too that occurs. 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 is 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 consists of four distinguishable but interacting steps: (i) hazard identification, (ii) dose – response assessment, (iii) exposure assessment and (iv) risk characterisation. Following these steps is essential in order to obtain information on which sound management decisions can be based. However, it must be stressed that the process is tedious, requires trained people and uncertainties in the calculations are an inherent part of the process. Nevertheless, a vast amount of published information, toxicity databases and human health risk assessment models are available that would overcome some of these difficulties.

The risk assessment methodology developed and proposed by the United States of America Environmental Protection Agency is detailed and well documented. The tables summarising the risk based consumption limits for different scenarios as well as the profile summaries for the target analytes are useful reference information that can be applied to real life situations. However, a major disadvantage of the information is that it is not compiled as a computer software package that performs the calculations and updates the tables automatically. Furthermore, if the computer software package has the capacity to link with other databases this would enable the risk investigator to obtain the most recent information and/or investigated other analytes not currently viewed as important.

In South Africa the risk assessment process related to the consumption of freshwater fish will be limited by the availability of local data and information and in many cases it would not be possible to perform the detailed assessments as undertaken by the United States of America Environmental Protection Agency. Furthermore, resources (financial and infrastructure) and shortage of skilled personnel would also limit the progress of assessing all the water bodies in South Africa. **To overcome some of the above-mentioned difficulties and to perform health risk based evaluations of chemical contaminants found in freshwater fish from South African systems it is recommended that the Risk*Assistant™ software package be applied (with the assistance from the competent personnel at the CSIR). The methodology described in this dissertation (Chapter 2 and Chapter 3, current section) should also be applied.**

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CHAPTER 4

AN INITIAL ASSESSMENT OF POSSIBLE HUMAN HEALTH RISKS ASSOCIATED WITH THE CONSUMPTION OF FISH FROM THE VAAL RIVER BARRAGE RESERVOIR AND THE KLIP RIVER, GAUTENG PROVINCE

CHAPTER 4

AN INITIAL ASSESSMENT OF POSSIBLE HUMAN HEALTH RISKS ASSOCIATED WITH THE CONSUMPTION OF FISH FROM THE VAAL RIVER BARRAGE RESERVOIR AND THE KLIP RIVER, GAUTENG PROVINCE.

4.1 INTRODUCTION

The discovery of gold in 1886 on the Witwatersrand was the first step in the discovery and utilization of the mineral wealth of the Pretoria-Witwatersrand-Vereeniging region. In the late 1800s and early 1900s one of the major problems experienced by the mining industry and local authorities was a reliable supply of water (Heath, 2000). This could be attributed to the fact that the main mining activities were approximately 60 km from the nearest major river (Vaal River) and that no large natural lakes are found in the area. For the Pretoria-Witwatersrand-Vereeniging region to develop its full economic potential it was therefore essential to ensure a reliable supply of water. To ensure that this region could rely on its water supply the Government sanctioned Rand Water (in 1903) to supply water to the local Authorities and the various mines. Rand Water was therefore responsible for the supply of water to the region - a task that since 1930 has been undertaken in collaboration with the Department of Water Affairs and Forestry. Subsequently, economic activities expanded in the region and today it is the main metropolitan, industrial and mining complex in South Africa.

Over the years the increase in demand for a reliable supply of water by the local authorities, mining industry and industry in general, in the Pretoria-Witwatersrand-Vereeniging and adjacent areas has resulted in the development of an extensive supply network. Source water is mainly obtained by Rand Water from the Vaal Dam (completed in 1938) and the Vaal River Barrage reservoir (completed in 1923) constructed in the Vaal River (Figure 4.1). Water is currently supplied to these storage reservoirs from three inter-basin transfers namely:

,

- Usutu River Transfer to Grootdraai Dam.
- Tugela River Transfer to the Sterkfontein Dam.
- Malibamatso River Transfer from the Katze Dam to the Axel River (Heath, 2000).

Initially the Vaal River Barrage reservoir (volume: 62 000 Mℓ; area: ± 680 ha; mean depth: 4m; length: 63 km) was the main reservoir of source water for Rand Water. However, most (97%) of the source water is now abstracted from the Vaal Dam. Nevertheless, the Vaal River Barrage reservoir is still an important emergency water source for Rand Water as well as a supply of water to property owners along its banks and downstream users. Furthermore, it has become a major recreational asset to the local communities, surrounding towns and cities. These activities include camping, canoeing, boating, picnicking, bird watching, water-skiing and fishing (DWAF, 1993; van Wyk, 2000). One of the main factors that would negatively impact on the recreational activities and the sustainable use of the reservoir is the deterioration in the water quality of the reservoir. The deterioration in the water quality can be attributed to the deterioration in the water quality of some of the rivers (for example: Klip River, Blesbokspruit, Taaibosspruit and Rietspruit) feeding into the Vaal River Barrage reservoir. In the catchment of

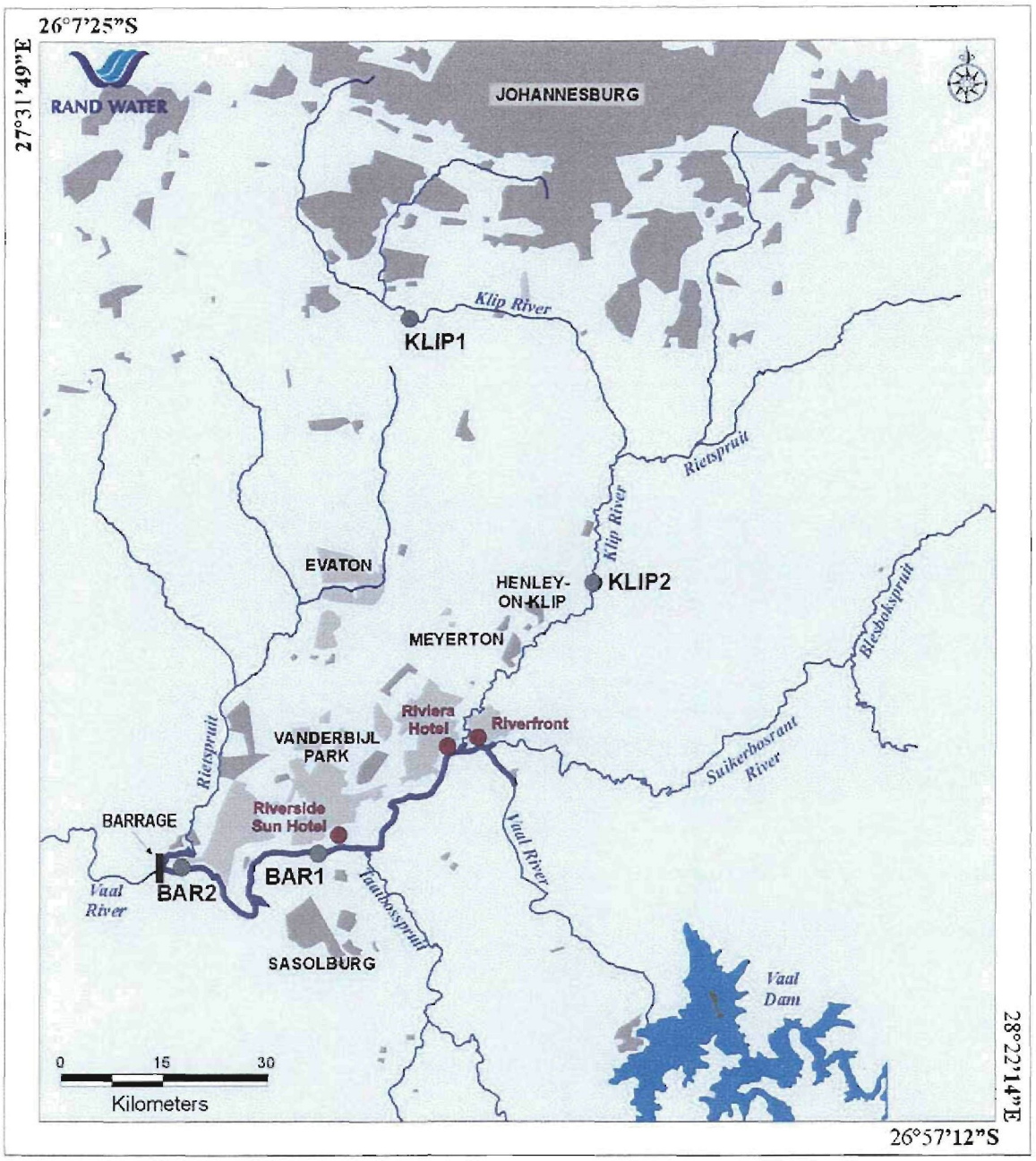

FIGURE 4.1: Location of sampling sites in the Vaal River Barrage Reservoir (BAR 1 & BAR 2) and the Klip River (KLIP 1 & KLIP 2).

these rivers urban run-off, run-off from informal settlements, poorly treated effluent from Waste Water Treatment Works, mine-water discharges and industrial effluents are all sources of Barrage river water contaminants (DWAF, 1993; Steward Scott Pulles Howard & de Lange, 1996; du Preez *et al.* 1998; Mulder *et al.* 1999a,b). These studies have reported several water quality constituents of concern, but ammonia, ortho-phosphate, sulphate, selected metals and low pH appear to be the most important. Bacteriological contamination of the river water is also a cause for concern. The deterioration of the water quality of some of the rivers feeding the Vaal River Barrage reservoir and that of the Vaal River Barrage itself, will not only impact on the aquatic system but will also influence human health. For example, the health of people may be at risk due to the bacterial contamination of the aquatic system and the consumption of contaminated fish by subsistence and recreational fisherman.

The concern regarding eating fish stems from evidence that bacterial or chemical pollutants may contaminate fish, which they accumulate and therefore pose a health risk to consumers. Fish may bioaccumulate organic or inorganic chemicals which enter the fish through the food ingested, non-food particles ingested (e.g. sediment), drinking water, the gills or the skin (Streit, 1998; du Preez, 1999). Within the body of the fish, the tissue and/or organs act as permanent or temporary dumping sites for these chemicals (Figure 4.2). However, the rate or degree of bioaccumulation in the tissue or organs may vary, for example, the level for lead bioaccumulation in *Barbus marequences* was found to be: vertebrae > gills > blood > bile > testis > kidney = liver > fat > ovaries > skin > muscle while that for zinc was skin > ovaries >liver > gills> vertebral > testis > muscle > blood > fat > bile (Seymore *et al.* 1995, 1996). Lipophilic contaminants, particularly organochlorine compounds are highly lipid (fat) soluble and have a pronounced tendency to accumulate in tissue or organs, (for example: body fat, gonads, liver) with a high lipid content (US EPA, 1997; du Preez, 1999). It must be stressed that fish have several routes, including the skin, gills, kidney, and egg deposition, to excrete contaminants. The bioaccumulation of a contaminant by fish is thus a function of the rate of uptake and elimination (excretion) from the body (du Preez, 1999).

The process of bioaccumulation of contaminants is influenced by many factors (Hellawell, 1986) and the extent of bioaccumulation of a specific contaminant by a fish depends on:

- Environmental variables. – Bioaccumulation can be affected by environmental conditions, the most important constituents being hardness of water, organic content, pH, temperature, turbidity and salinity.
- Interactions between substances. – If two or more pollutants are present, the effect can be additive, antagonistic or synergistic.
- Uptake and excretion rates. – The rates of uptake and loss of a pollutant determine the net concentration of the pollutant or its metabolites in the fish. The concentration could reflect prevailing environmental levels with rapid accumulation and low excretion rates.
- Trophic status of the bioaccumulator. – High residue levels, (for example for organochlorine pesticides) in species from higher trophic levels, are often the result of bio-magnification.
- Physiological condition of the bioaccumulative indicator. – Changes in lipid (fat) storage, feeding activity or reproduction can vary with the season. Bioaccumulation of organic compounds specifically is affected by the fat content of the organism or tissue. Fish species with higher lipid levels would therefore tend to have higher levels of lipophilic contaminants.
- Age and size of the bioaccumulative indicator. – Some authors have confirmed the effects of size and age on bioaccumulation. The effects could be explained by different mass/surface area rations and long-term accumulation with limited excretion.

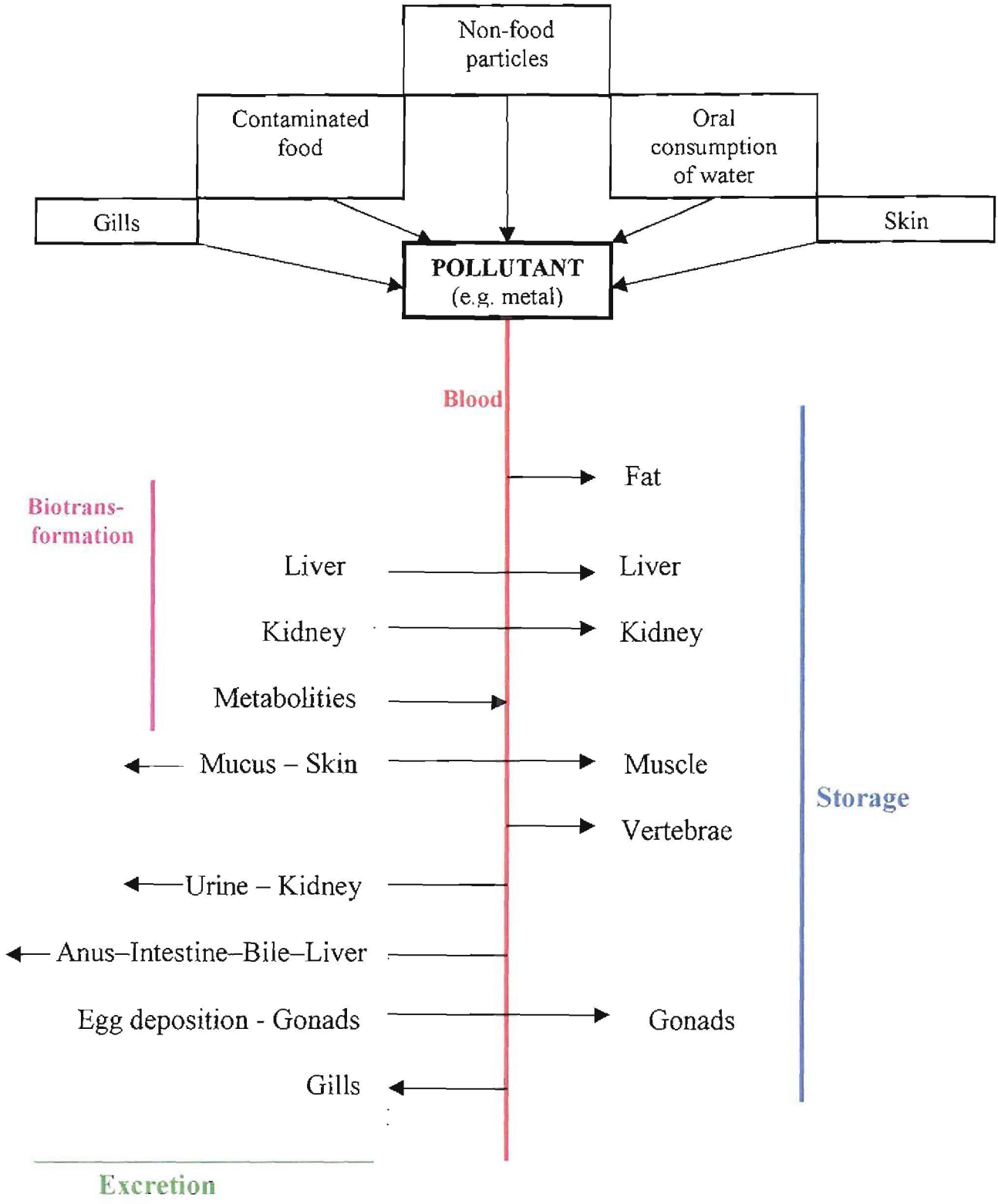

FIGURE 4.2: A summary of the possible pathways of a metal in fish after its uptake [adapted from Heath, (1987) and du Preez, (1999)].

Nevertheless, fish have the potential to bioaccumulate contaminants with varying chemical structure and toxicity that may pose a health risk (for example, cancer, chronic systemic effects, developmental effects, reproductive effects, etc.) to consumers (US EPA, 1997).

In this section of the dissertation the concentrations of selected metals in the fillets of fish from the Vaal River Barrage River and the Klip River (a main source of polluted water, especially during the dry season) were assessed. The main objectives were to:

- *Apply and evaluate the sampling and analysis protocol proposed in Chapter 2 of this dissertation.*
- *Apply and evaluate the risk assessment methodologies proposed in Chapter 3 of this dissertation.*
- *Assess the present status of metal levels in the fish and make recommendations for further investigations in the study area.*

4.2 MATERIALS AND METHODS

4.2.1 Study sites

Data from previous surveys (May 1997 and November 1997) at two sites in the Vaal River Barrage reservoir were obtained and used in the present study (Groenewald, 1999). A field survey was also undertaken during May 1998 to collect fish from the Klip River, one of the main rivers flowing into the Vaal River Barrage (Figure 4.1). Therefore, data from fish collected at the following sites were used in the present study (Figure 4.1):

- **KLIP 1:** Klip River downstream of the confluence of the Klipspruit and effluent discharge point of the Olifantsvlei Waste Water Treatment Works. The fish were collected upstream of the small weir at this site.
- **KLIP 2:** Klip River at Henley-on-Klip weir of the town Henley-on-Klip. Fish were collected upstream of the Henley-on-Klip weir.
- **BAR 1:** Vaal River reservoir at the Abrahamsrust Pleasure Resort.
- **BAR 2:** Vaal River Barrage reservoir at the reedbed island at barrage wall.

4.2.2 Field sampling

Two freshwater fish species were collected, *Labeo umbratus* (moggel) and *Labeo capensis* (Orange River mudfish) by Groenewald (1999) at the sites in the Vaal River Barrage (Sites: BAR 1 and BAR 2). During the present study fish were also collected by means of gill nets (40 mm to 150 mm stretch mesh size) from the Klip River. The nets were placed before sunset and the fish removed the following day at sunrise.

After capture the selected fish were transferred to a holding tank filled continuously with water from the site. Before dissecting a fish, the fish was rinsed in water from the site to remove any foreign material from the body surface. The mass, total length (see Chapter 2: Figure 2.1) and sex (after dissection of tissue) of each fish was recorded. The fish was then killed by a hard blow on the head and a severing of the spinal cord behind the head. Dissection was done on polyethene dissection boards, using high quality stainless steel dissection tools whilst wearing surgical gloves (Heit and Klusek, 1982; US EPA, 1995; see Chapter 2: Section 3). All dissecting instruments and glass bottles were pre-washed as described in Section 4.2.3. Muscle tissue (skinless) was then removed from each specimen, placed in pre-washed glass bottles and

frozen until further analysis in the laboratory could be performed (see Chapter 2: Table 2.6 and Table 2.7). To prevent contamination the work surface and dissection instruments were cleaned before the next fish was dissected (see Chapter 2: Section 3).

4.2.3 Laboratory procedures

Before use, all glassware was washed and then soaked in a 2% Contrad (Merck Chemicals) soap solution for 24 hours. After 24 hours in the soap solution it was rinsed with deionised water and acid-soaked in a 1M hydrochloric acid (HCL) solution for another 24 hours. Thereafter it was again rinsed with deionised water (Griesy & Weiner, 1977; US EPA, 1995; see Chapter 2: Table 2.7). All other instruments and dissection surfaces were pre-washed in tap water, then washed with a 2% Contrad soap solution, then rinsed with deionised water, and thereafter acid rinsed (1M HCL) and finally rinsed in deionised water.

Metal concentration analysis

At the laboratory the samples were thawed and approximately five grams of each sample were placed in a 100 mℓ Erlenmeyer flask and dried in an oven at 60 °C for a period of 48 hours in order to determine the moisture content of each sample. Ten millilitres concentrated nitric acid (55%, Merck Chemicals) and five milliliters concentrated perchloric acid (70%, Merck Chemicals) in a 2:1 ratio were added to individual samples for digestion. Digestion was performed on a hot plate at 200 to 250 °C for approximately four hours or until the solution was clear (Van Loon, 1980). After digestion was completed each sample was allowed to cool before being filtered through an acid-resistant 0.45 µm filter paper by using a vacuum pump. Each sample was then made up to 50 mℓ with deionised water and stored in pre-washed 100 mℓ amber glass bottles.

The flame analytical method (Varian Atomic Absorption Spectrophotometer, Spectra AA-10) was used to determine the metal concentrations (aluminium, cadmium, copper, lead, manganese, nickel, strontium, zinc) in the muscle tissue of the fish (Varian, 1989). Analytical standards were prepared from Holpro stock solutions. Additional quality assurance steps included the use of standard reference material (Cod muscle – CRM 422: supplied by Industrial Analytica, South Africa) and the analysis of triplicate acid blanks. Furthermore, only analytical grade chemicals were used.

The metal concentrations in the different muscle tissue samples were calculated as:

$$\textbf{Metal concentration (µg/g dry mass)} = [\textbf{AAS reading (µg/mℓ) / dry sample mass (g)}] \times \textbf{sample volume (50 mℓ)} \qquad (4.1)$$

The metal concentrations were then expressed as µg/kg wet mass using the following equation:

$$SMC_{wet} = (100 - SMC\ \%) \times SMC_{dry} \times 1000 \qquad (4.2)$$

where:

- SMC_{wet} = Metal concentration of the muscle tissue expresses as µg/kg wet mass tissue.
- SMC **%** = Percentage moisture in the tissue sample as determined by oven drying at 60°C for 48 hours.

SMCdry = Metal concentration of the tissue expressed as ug/g dry mass tissue and derived from Equation 4.1.

4.2.4 Statistical procedures and data processing

General statistical analyses were performed using the Graph Pad Prism (version 3.0) computer software package. The different statistical analyses were only performed on certain data because fish capturing success varied and it was not always possible to obtain the same species of a specific size and sex at each locality. As this preliminary survey was not designed to investigate difference between species or intra-species differences (sex, size), data for all the individuals captured at a specific site were grouped in the statistical analysis. The influence of these population variables as well as seasonal variation for the data from Vaal Barrage sites (sites: BAR 1 and BAR 2) are being assessed by Groenewald (1999). Basic summary statistics are, however, given for each species collected at the different localities.

In the statistical analysis all data points were used including possible outlier values. This approach was used as it was assumed that all data points are real (described variation in metal concentrations in the fish) and are not a result of sampling and/or analytical errors. Normality tests revealed most of the grouped data for each site deviated significantly from a Gaussian distribution using the Kolomogorov-Smirnov test. Subsequently, distribution free statistical tests (non-parametric tests) were used as they make fewer assumptions about the distribution of the data. The non-parametric Kruskal-Wallis test was used to test differences between three or more groups of unpaired data sets. The Dunn's multiple comparison tests to identify which groups are significantly different followed this test.

4.2.5 Risk assessment

The different steps of the risk assessment process are described in detail in Chapter 3 of this dissertation. Therefore, only a brief description of the procedures followed in the present health risk assessment of the selected metals in the fillets of fish from the Klip River and Vaal River Barrage is given. Mrs. Bettina Genthe of the CSIR (Division of Water Environment and Forestry: Stellenbosch) assisted with the risk assessment process by interrogating the toxicity databases and applying the Risk*Assistant™ software package to fish chemical contaminant data.

Hazard identification and dose response assessment

Toxicity databases such as Agency Toxic Substances and Disease Registry (ATSDR, 1999), Integrated Risk Information System (IRIS, 1999) and Toxicology Excellence for Risk Assessment (TERA, 1999) were used to evaluate the toxicity and carcinogenicity of the various metals.

Exposure Assessment

Only one route of exposure was included in this risk assessment, namely ingestion offish fillets. However, various exposure scenarios were included in the health risk assessment as summarised in Table 4.1. Assumptions that both adults and children would be exposed to metals in fish were made. Since no socio-demographic information (age, sex) of the consumers of freshwater fish from the Klip River and Vaal River Barrage reservoir is available, it was assumed that adults and children consume fish at different frequencies and meal sizes. This would provide an indication of the potential range of health risks as it was assumed that different amounts of fish