Republic of South Africa

NATIONAL TOXICITY MONITORING PROGRAMME:

REPORT ON PHASE 3: PILOT IMPLEMENTATION AND TESTING OF THE DESIGN

Department of Water Affairs and Forestry
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EXECUTIVE SUMMARY

Background

The pilot implementation of the national toxicity monitoring programme, which ran between June 2006 and October 2007, is the third phase of the design of this programme. The aim of the this phase was to test the design and to establish the optimal sampling frequencies for various selected constituents, which included: aldrin, some arochlors, some triazine herbicides (simazine, atrazine, terbutylazine) lindane, *cis*- and *trans*- chlordane, DDT and its metabolites (DDE, DDD), dieldrin, endrin, endosulfan congeners, heptachlor and its breakdown product, mirex, monocrotophos, some alkyl phenols, a variety of phthalates, toxaphene in addition to toxicity tests using *D. rerio*, *D. pulex*, *P. reticulata*, *S. capricornutum* and an engineered *V. fischeri* enzyme inhibition test.

In order to bring about some saving to a project that would have cost an estimated R3.3M, the sampling between the NTMP, the NRMP and another ad hoc project in the Jukskei river were consolidated. In addition, the sampling frequency at some points was scaled down during base flow conditions resulting a project cost of just more that R1M. Sampling points were selected in the Jukskei River at Marlboro, Midrand and N14 – (representing urban run-off as well as municipal sewage treatment works discharge), in the Klip River (representing a combination of industrial discharge and metropolitan run-off) in Gauteng, in the Kleinspruit in Mpumalanga (representing run-off from an industrial town) and in the Jagspruit (representing an agricultural, pre-mining baseline water constituency).

Results

Despite several sampling, infrastructural and laboratory analytical problems, the data collected so far already yields information that was not available before. The interpretation of the data is not unequivocal as yet due to the non-availability of accepted assessment criteria. However, based on proposed criteria these sites were assessed as shown in Tables A and B below.

It was found that generally the response, both in terms of chemical analysis and toxicity, was low. The triazine herbicides and phthalates occurred most frequently among the chemicals tested for. The *S. capricornutum* test displayed stimulation of growth rather than inhibition, indicating an enriched growth environment. Although some inhibition and mortality was observed for the water fleas and fish used in the tests, this was relatively rare.

Table A. An assessment of ecological status based on tentative guidelines for the some of the Stockholm Convention persistent organic pesticides.

Toxicant	Marlboro	Midrand	N14	Kleinspruit	Klip River	Jagspruit
Aldrin	Fair	Natural	Natural	Natural	Natural	Natural
Atrazine	Natural	Good	Natural	Good	Natural	Natural
Chlordane	Natural	Natural	Natural	Natural	Natural	Natural
DDT	Natural	Natural	Fair	Poor	Fair	Fair
Dieldrin	Natural	Natural	Poor	Natural	Natural	Natural
Endosulfan (α+β)	Natural	Natural	Good	Natural	Natural	Natural
Endrin	Natural	Natural	Natural	Natural	Natural	Natural
Heptachlor	Poor	Natural	Natural	Natural	Natural	Natural
Hexachlorobenzene	Natural	Natural	Natural	Natural	Natural	Natural
Lindane	Natural	Natural	Natural	Natural	Natural	Natural
Mirex	Natural	Natural	Poor	Natural	Natural	Natural
Monocrotophos	Natural	Natural	Natural	Natural	Natural	Natural
Simazine	Natural	Natural	Natural	Natural	Natural	Natural
Toxaphene	Natural	Natural	Natural	Natural	Natural	Natural

The high occurrence of non-detects, i.e. test results that showed that the testing methodology was non sensitive enough to respond to the amount of material in the sample, resulted in difficulty in determining the optimal sampling frequency.

Table B. A toxicity assessment of the selected sites.

Site	Typically	At worst
Marlboro	Good	Fair
Midrand	Good	Good
N14	Good	Poor
Kleinspruit	Good	Good
Klip River	Good	Good
Jagspruit	Good	Good

The results (toxicity or toxicant) might be low due to any of the following reasons:

1) There are really very little of the toxicants present in the system,
2) The toxicants are in reality higher than the results suggests but they are not really found in the water column but rather in the sediment or biota,
3) The toxicants really are more abundant but they are in different locations, or
4) The occurrence of toxicants is highly episodic and the current frequency of sampling is too low to detect all but the occasional peak.
5) The testing technology is not selective enough to assess the issues being addressed

The current design of the NTMP does not address 2) and there is insufficient data to pronounce on 3 and 4). Although option 1) may be true, a more conservative null hypothesis would be that the selected toxicants are present at significant, if not high, levels. This means that some design changes for the NTMP are necessary which must address:

Sampling media

At present only the water column is addressed. The interconnectedness of water resource compartments such as the water column, the transported and precipitated solids and the biota requires an extension of the design. It is recommended that trace organic content of sediment and biota be investigated as a matter of urgency. This investigation should establish the relevant methodology, the optimal sampling frequency and the most appropriate environmental media to estimate the immediate and long term status. In the longer term a suitable water quality model should be developed to aid interpretation. A separate riverine solids monitoring programme is advised.

Choice of sampling sites

The choice of sampling sites will remain a subjective exercise for the foreseeable future. Ideally a national survey should establish the areas in which high toxicant concentrations or high toxicity is found. At present these sites are selected based on perceived potential for contamination. However, the sites need to be characterized carefully based on sound insight regarding their flow and concentration patterns. It is conceivable that different sites may yield different toxicants of concern and distinctive toxicity patterns. Consequently the next step would be to perform an intensive assessment at each proposed site.

Frequency of sampling

One of the issues that need attention in site characterization is higher confidence characterization of the statistical distribution characteristics of toxicants and toxicity at selected sampling sites. This will require high frequency sampling for short periods of time. Based on hypothesised mechanisms of transport of toxicants to a given site (e.g. wash-off and/or dilution) a sampling strategy suitable to characterize this mechanism can be implemented for relatively short periods.

Trace elements
The focus in the original design of the NTMP was on toxicity and selected trace organic toxicants. There is at present no systematic study on the occurrence of trace elements and particularly heavy metals in our river systems. It is therefore proposed that V, Mn, Ni, Cu, Zn, As, Se, Sr, Cd, Sn, Sb, W, Hg, Pb, Bi and U(all dissolved and total) as well as Cr(VI) be as well as the necessary major cations, anions and physico-chemical characteristics of the water necessary to interpret the results be included in the NTMP variables. Until such time as the South African Water Quality Guidelines have been updated, the relevant ANZECC guidelines can be used if required.

Research and development work required
Ideally, rapid and sensitive methods that yield continuous responses to contaminants should be considered. Focussed investigations that pronounce on the most suitable methods and their interpretation in terms of the national classification system are required as a matter of priority. In this process the focus should ideally be on methodology that is viable for in-house application (to the extent possible) and, as a corollary, there should be a strong focus on in-house capacity building. At the same time it is vitally important that scientific credibility be given the highest priority.

The investigation into- and development of a practical integrative sampling device, which reflects an integration of the recent exposure history at a site and which reflect the toxicant load at the site, should be given serious attention as this will help address the problem of missing toxicant peaks in the sampling regime.

Conclusion
There is no other data currently collected by DWAF either directly or through other agencies that can yield the same insights into the status of toxicants and toxicity in river systems. Even at a cost of more than R1 M, the expenditure thus far is justifiable. While development work is under way, attention should be given to selecting suitable sites to assess the toxicity status of the national water resource and these should then be systematically characterized.

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Term	*Explanation*
Ecological Water Quality Reserve	The water quality input to the Reserve process. The Reserve is the quantity, quality, timing and biotic and abiotic habitat requirement to maintain a river in a given class.
LC50	A term used in toxicology to denote the concentration of a substance where 50% of the exposed population dies
LOEC	A term used in toxicology to denote the lowest concentration where an adverse effect can be resolved from the control population with a given degree of statistical significance
NOEC	A term used in toxicology to denote the highest concentration where no adverse effect can be resolved from the exposed population with a given degree of statistical significance
WMA	Water management area
WMS	Water management System, a data base of South African water quality data in surface and ground water.

Chapter 1 Background

The National Water Act (Act 36 of 1998) requires the Minister to establish national monitoring and information systems that monitor, record, assess and disseminate information on water resources. To comply with this requirement of the National Water Act as well as the National Water Resources Strategy, the Department of Water Affairs and Forestry (DWAF) has embarked on the development of a National Toxicity Monitoring Programme (NTMP) as one of a series of national monitoring programs that are intended to give effect to the NWA mandate.

The NTMP was designed in response to increasing local and international concerns about the detrimental effects of toxicants that are being released into the environment and to address the current lack of a coherent source of information on the occurrence of toxic substances in South African water resources. The project was initiated in 2002, incidentally the same year that South Africa signed the Stockholm Convention on Persistent Organic Pollutants (POPs), which came into force on 17 May 2004. Obligations for South Africa in terms of the Convention include the development and maintenance of appropriate information dissemination programmes (Article 10) as well as the undertaking of research on all matters relating to POPs. This includes monitoring, socio-economic impacts and release reduction (Article11). In order to meet the information requirements of the Stockholm Convention regarding the presence of POP's in fresh surface water resources, the monitoring of POPs (excluding dioxins, fire retardants, furans and hormones) were included as variables of concern in the conceptual design of the NTMP.

The NTMP project comprises of the following four distinct phases:

Phase 1: Needs assessment (completed in March 2003);
Phase 2: Development of implementation plan (completed in March 2006);
Phase 3: Testing and refinement of NTMP design and implementation plan;
Phase 4: Implementation and evaluation of the monitoring programme.

This document reports on the Phase 3: Testing and refinement of NTMP design and implementation plan

Chapter 2 Introduction

In Phases 1 and 2 of the design of the NTMP it was concluded that:

- Both toxicants (limited to the selected POPS) as well as toxic effects should be monitored in the NTMP, but the primary focus is on effects, i.e. "toxicity" in the broader sense of the word.
- Although a wide range of toxicity tests is available to measure the effects on organisms, many are relatively costly and difficult to apply and interpret. For this reason that it was decided to limit the programme to only four widely used toxicity tests and the monitoring of toxicants to POPS (excluding the chlorinated-dibenzodioxins and -dibenzofurans). Two additional tests currently being used internationally, although locally limited, may be phased-in.
- The monitoring programme should be designed, planned and executed in a modular way. This will make the NTMP more cost effective, simplify project planning, will be conducive to adaptive management and allow greater depth of understanding.
- Although it would have been more economical (in terms of cost and time) for DWAF to follow a simultaneous approach in the design of monitoring systems for surface water, groundwater and estuaries, they have fundamentally different characteristics. The NTMP would thus initially focus on fresh surface water systems only.
- Due to the high cost of the NTMP, the programme would initially concentrate on priority areas or hot spots only.
- To accommodate the Stockholm Convention the NTMP would include the so-called POP's list with the exception of dioxins, fire retardants and furans (due to cost and capacity implications). Substances with endocrine activity, although important would not be included in the pilot implementation based on their endocrine activity. The list of toxicants for implementation into the pilot scale of the NTMP was as follows:
    1. Aldrin
    2. Chlordane
    3. DDT and selected breakdown products (DDD and DDE)
    4. Dieldrin
    5. Endosulfan, (α-endosulfan, β-endosulfan and endosulfan-sulphate)
    6. Endrin
    7. Heptachlor
    8. Hexachlorobenzene
    9. Lindane and selected breakdown products (α-BHC, γ-BHC and δ-BHC)
    10. Mirex
    11. Monochrotophos
    12. Four PCB congeners: 2',5' dichloro-4-hydroxybiphenyl; 2',5' dichloro-3-hydroxybiphenyl; 2',4',6' trichloro-4-hydroxybiphenyl and 2',3',4',5' tetrachloro-4-hydroxybiphenyl
    13. Toxaphene
    14. Three triazines (atrazine, simazine and terbutylazine)

- The suite of toxicity tests that have been selected for inclusion are:
    - The *Vibrio fischeri* bacterial bioluminescence inhibition test
    - An algal 24-well microplate growth inhibition (AGI) test
    - A *Daphnia pulex reproduction* test (lethality and sub-lethality – this test will also be phased in gradually over the 2 year implementation period)
    - 96 hour acute *Poecilia reticulata* (Guppy) test

  - A semi-static *Brachydanio rerio* [Zebra fish] development test (will be developed/optimised and validated at RQS laboratory and will be phased in gradually over the 2 year implementation period)
  - The recombinant yeast (hER) method will be developed/optimised and validated at RQS laboratory (this test will be phased in gradually over the 2 year implementation period)

- Additional developmental work is necessary to address issues such as:
  - Human cell line test in the NTMP
  - Possible use of commercially available test kits for use in the NTMP
  - Which phase of the aquatic environment e.g. water column sediment or both would be the most appropriate to monitor
  - Analysis of aquatic biota to investigate occurrence of POPS in the aquatic environment
  - Building the capacity (in terms of human resources as well as laboratory capacity) required to support full scale implementation of the NTMP
  - Conducting a needs analyses to determine the requirements to support the design of the groundwater and estuarine components of the NTMP

It was clear from the outset that designing a programme of this nature would entail significant difficulties and that the pilot implementation would be needed to address some of these. The aim of Phase 3 was to test the conceptual design of the NTMP for a period of 2 years in order to be able to refine and revise the NTMP Implementation Manual if necessary before the final phase, namely full scale implementation commences.
The specific objectives of the pilot implementation were to test the following components of the design:

- Sampling site selection
- Sample collection and handling (including infrastructure and logistics)
- Sample analyses
- Data capture and storage
- Assessment of monitoring results
- Statistical design including sampling frequency
- Information generation and dissemination
- Verification of the capacity building plan
- Quality Assurance procedures

The following tasks will be performed to reach the above objectives:

Task 1: Monitoring site selection
The macro-sites that were provisionally selected (according to the macro site selection criteria) are in the following Water Management Areas (WMA's) (Figure 1):

- The Crocodile West/Marico WMA in the Jukskei River: 2 sites; 1 below a residential area including a squatter camp, and 1 below the Johannesburg Northern Treatment Works
- Middle Vaal WMA: 1 site in the Waterval river in Secunda and 1 site in Orkney at the intake of Midvaal Water
- The Limpopo WMA: 1 site in the Luvuvhu river (this links up with the EDC programme of the WRC)

The micro location (according to the micro site selection criteria proposed in the conceptual design) of these sites were to be determined in Phase 3.

Deliverable of Task 1: a) A map indicating the potential high risk areas within South Africa (where toxicity may be expected in surface water resources) and the macro sites within each of the high risk areas and b) Verification of the macro and micro sampling site selection criteria as proposed in the NTMP conceptual design.

Task 2: Sampling Frequency

Varying the sampling frequency at the selected sites would test the statistical design of the programme. It was proposed that at the sites in industrial areas sampling be done at a high frequency (weekly) for 4 months (2 months in the rainy season and 2 months in the dry season). Monthly sampling was proposed for the remaining 8 months. The proposed sampling frequency and the number of samples are as follows:

Crocodile West Marico WMA; Jukskei River:

- Industrial site:	weekly for a 4 month period	= 16 samples
	monthly/8 month period	= 8 samples
-JHB Northern Works	weekly/4 month period	= 16 samples
	monthly /8 month period	= 8 samples

Middle Vaal WMA:

- Waterval	weekly/4 month period	= 16 samples
	monthly/8 month period	= 8 samples
- Orkney	weekly/4 month period	= 16 samples
	monthly /8 month period	= 8 samples

Limpopo WMA

- Luvuvhu	monthly/12 month period	=12 samples

Deliverable of Task 2: Testing of the statistical design (including Prof Schüürmann's model [Chemprop]) of the NTMP as well as logistics with respect to sample collection and transport in the WMA's that are not close to the laboratory facilities. The necessary corrections will be to the statistical design as well as data management and storage component in the Implementation Manual.

Task 3: Sample analysis

All samples will be analysed by the laboratories at the RQS. Samples will be analysed for the list of toxicants (POPs) and the toxicity test performed as described earlier in this chapter.

Deliverable of Task 3: Testing of the feasibility of the data acquisition (including the Quality Assurance and Control) component of the NTMP design including verification of the analytical procedures/tests. The semi-static *B. rerio* test and hER test will be performed as development work and will be phased in gradually over the implementation period. The same phased-in approach applies to the chronic *D. pulex* test. Revision of concepts/procedures and changes to the design and the Implementation Manual where necessary.

Task 4: Data management and storage

All the data generated during Phase 3 were to be captured and the data transferred to the WMS.

Deliverable of Task 4: Testing of the data management and storage component of the conceptual design. Changes could be made to the design and the relevant section in the Implementation Manual revised if necessary.

Task 5: Information generation and dissemination

The data were to be extracted from the WMS, analysed and assessed against the guidelines for Domestic Use and Aquatic Ecosystem Health and procedures contained in the Implementation Manual. Prototype reports would then be generated and distributed to representatives of the primary and secondary clients for comments.

Deliverables of Task 5: Testing of Information Generation and Dissemination component of the conceptual design and revision (where necessary) of the procedures contained in the Implementation Manual.

Task 6: Capacity building-plan and cost estimate of full-scale implementation of the NTMP.

The capacity building plan and the cost estimation model that was developed in Phase 2 will be verified in terms of the actual resources (human, financial and physical) that are required to support the pilot implementation (Phase 3) of the NTMP.

Deliverables of Task 6: Revision of the Capacity Building Plan and refinement of the cost estimation model for full-scale implementation of the NTMP. This information will serve as an input into the RQS' 5-year strategic business plan

Task 7: Development work

The development work that would be necessary to support the sustainable implementation of the NTMP will be identified and initiated including:

- Determining which phase of the aquatic environment e.g. water column, sediment, biota tissue or both would be the most appropriate to monitor
- Investigating the usefulness of other toxicity tests such as the inclusion of tests employing the use of human cell lines in the NTMP
- Possible use of commercially available test kits for use in the NTMP [as an alternative to lab culturing of organisms in order to help address the shortage of equipped laboratories]
- Analysis of aquatic biota to investigate occurrence of POPS in the aquatic environment
- Semi-static *Brachydanio rerio* [Zebra fish] test will be done as development work in parallel with *Poecilia reticulata* (guppy) test at least for duration of pilot implementation phase and will be phased in
- hER method will be developed/optimised and validated at RQS laboratory while being outsourced during the Pilot phase implementation
- Building the capacity (in terms of human resources as well as laboratory capacity) required to support full scale implementation of the NTMP
- Conducting a needs analyses to determine the requirements to support the design of the groundwater and estuarine components of the NTMP

Deliverable Task 7: Identification of developmental work that needs to be done to support the sustainable implementation of the NTMP. The development of the ToRs to initiate the identified projects. Co-ordinating the contributions of other role players such as the WRC that will assist DWAF with the necessary development work.

Final deliverable of Phase 3: A revision of the conceptual design of the NTMP and the Implementation Manual as well as a refined cost analysis for full-scale implementation of the NTMP.

This document constitutes the final deliverable of Phase 3 and comments on all the relevant tasks above. The structure of the report follows the task structure above to facilitate finding the relevant data.

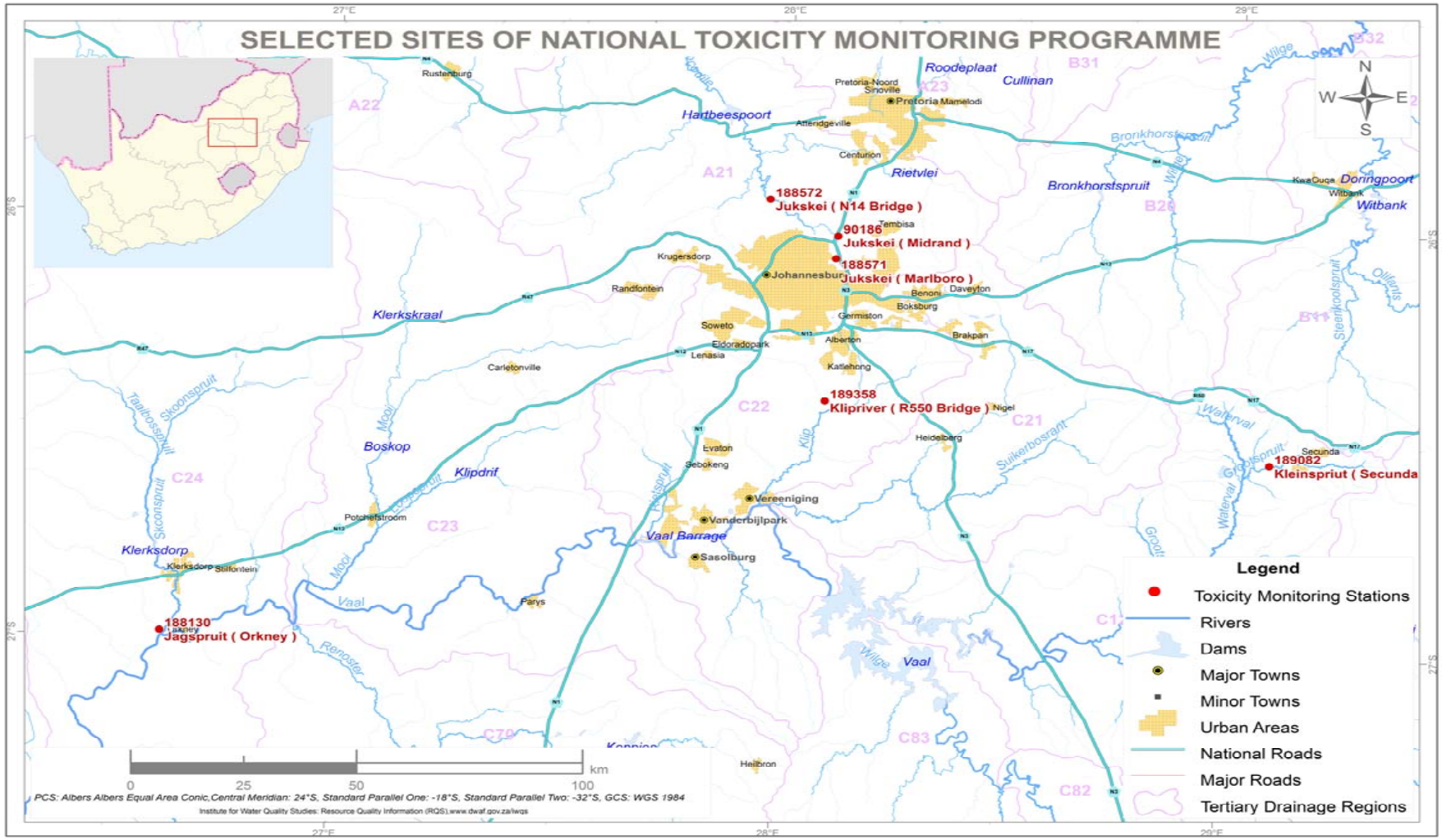

Figure 1. Geographic location of sampling sites used in the pilot implementation of the NTMP

Chapter 3 Results and Discussion

The results of phase 3 of the NTMP are presented in terms of the task outcomes as described in Chapter 2. The discussion following represents all the most important conclusions from this phase.

Task 1 Monitoring site selection
The sites proposed and those finally used are shown in Table 3.1. Figure 1 shows a map indicating the sampling sites used. The sites finally selected (with the exception of the Jagspruit near Orkney) represent sites that one could reasonably have expected to find some toxicants.

Table 1 Monitoring sites selected for Phase 3

WMA	Proposed	Used	Comment
Crocodile West /Marico	Jukskei below major industrial activities	Jukskei Upstream of Marlboro Drive Bridge (WMS point 188571, Figure 2)	Site downstream of both a major industrial complex and the township of Alexandra. In 2008 sampling was curtailed due to Gautrain construction work.
	NA	Jukskei at the R101 crossing in Midrand (WMS point 90186, Figure 3)	About 5km down stream of the site above. Except for sand mining and some storm water discharge this site has no other new impacts and should generally give an indication of the temporal/geographic change in variable levels.
	Jukskei below Johannesburg Northern works discharge	Jukskei at N14 Bridge (WMS point 188572)	About 1000m downstream of the sewage works discharge
Middle Vaal	Waterval River	Kleinspruit at the R546 bridge (WMS point 189082, Figure 4)	The selected site is closer to the industrial complex at Secunda than the Waterval River.
	Orkney	Jagspruit at the R524 bridge (WMS point 188130)	The selected site is just downstream of proposed mining development – this will provide a baseline to monitor possible impacts
	NA	Klip River downstream of the R550 bridge (WMS point 189358, Figure 5)	This site represents drainage from a large part of southern Johannesburg including several industries.
Limpopo	Levhuvhu River	Not used	The effect of DDT spraying in the Venda area is monitored at this site by teams from Universities of Pretoria, Johannesburg and North West with particular emphasis on estrogenic activity. Not easily accessible.

The sites on the Jukskei River have been selected because they form part of another project aimed at establishing the profile of organic contaminants in a semi-urban stream subject to industrial discharge, urban run-off and sewage discharge. This

project has its own ToR and the NTMP pilot implementation was piggy-backed on this project in order to save expenditure. At the same time the variable to be analysed for in that project were adjusted to provide the necessary input for the NTMP. The site on the Jagspruit was sampled in conjunction with the National Radioactivity Monitoring Programme (NRMP) in order to save cost. Due to the local nature of phase 3, a national map can not be produced at this stage – this can be done only when the full implementation of the NTMP commences.

Figure 2. The sampling point at Marlboro on the Jukskei River

Figure 3. The sampling point at the R101 crossing near Midrand on the Jukskei River.

Figure 4. The sampling point at R 546 road bridge on the Kleinspruit near Secunda

Figure 5. The sampling point near the R550 on the Klip River.

Task 2: Sampling Frequency

The sampling frequency used during this pilot phase is shown in Table 2. Sampling commenced on 26 July 2006 at the exclusive NTMP sites with monthly sampling and concluded in October 2007.

Table 2. Sampling frequency used in phase 3.

WMA	*Sampling site*	*Sampling frequency*	*Expected number of samples*
Crocodile West	MARLBORO (WMS point 188571)	Weekly	100*

/Marico	MIDRAND (WMS point 90186,)	Weekly	100*
	N14 (WMS point 188572)	Weekly	100*
Middle Vaal	KLEINSPRUIT (WMS point 189082)	Monthly (7/06,8/06, 9/06, 10/06, 3/07, 4/07, 5/07 2 weekly (11/06, 12/06, 6/07 7/07 Weekly (7/06, 1/07, 2/07,7/07)	31
	JAGSPRUIT (WMS point 188130,)	As for Kleinspruit	31
	KLIP RIVER (WMS point 189358,)	As for Kleinspruit	31
TOTAL NUMBER OF SAMPLES			**393**

*For period June 2006 to October 2007

Sampling routes

Two sampling routes were established for the pilot phase: one sampling the Middle Vaal and one sampling Jukskei (the latter is an existing project for which the sampling schedule was simply continued). The Middle Vaal route spanned three provinces (North West, Gauteng and Mpumalanga) with a round trip of about 640 km taking 10-12 hours. The Jukskei route concentrated on Gauteng with a round trip of about 210 km taking 4 to 6 hours. Travelling time and traffic congestion proved to be major factors on both routes.

Establishing the minimum frequency: hypothesis testing (HT) approach

The easiest strategy for assessing the frequency of sampling the design would be as follows:

1. Pooling the high frequency and low frequency samples at each site would yield a fair indication of the variability at each site. (Even though the sampling frequencies differ, it was assumed that flow would play a dominant role in the transport of contaminants in the river. Since both high and low flow conditions were sampled with high frequency there should be no significant bias in the data record.)
2. A distribution would then be fit to the data and the location and scale parameters determined (each with a know confidence interval) using maximum likelihood methods (the use of maximum likelihood methods would normally be necessary because a certain number of analyses are likely to lower than a given method detection limit, rendering regression fitting useless).
3. Using a Monte Carlo approach, bootstrap sampling[1] will produce a number of data sets composed of measurements at lower frequencies. By fitting the same type of distribution used for the pooled data to the re-sampled data would produce a new set location and scale parameters (with attendant confidence intervals) – one for each frequency selected.
4. Each of the records generated above can then be tested for acceptance/rejection of the null hypothesis that the chosen distribution parameter of the pooled set is not significantly different from the same parameter for the Monte Carlo set. The lowest frequency at which the null hypothesis is not rejected is the lowest feasible frequency for that particular site.

[1] Bootstrapping refers to a sampling scheme for a data set where repeated samples are drawn randomly from a data set in such a way that it is possible to draw the same sample more than once. It is equivalent to 'sampling with replacement'.

For each variable of interest in the record, the strategy above is critically dependent on: a) not having sampling (or analytical) bias in the data record, b) having a sufficient number of samples testing above the detection limit to ensure that the confidence intervals are narrow enough that the null hypothesis can test negative at some frequency (i.e. the power of the test is sufficient), and c) having a sufficient number of analysed samples at a frequency that exceeds the expected maximum frequency.

Evaluating the sampling frequency by the HT methodology above proved to be a significant problem in the Middle Vaal where data availability was limited to the NTMP samples. A number of problems were experienced particularly by the organic laboratory. This meant that all the samples were not necessarily analysed or not all variables were analysed for on each sample. Many compounds were not detected at all. The result is that the conditions for the use of the HT methodology were not met. An additional problem arose in that there is no specific indication on what the variability for each compound is. One therefore has to assume (based on nothing but happenstance) that weekly sampling represents a higher frequency than would be needed. While this may be true under low flow conditions, there is no guarantee that under high flow conditions this assumption would hold. (In support of this view one could note that "flash floods" occur quite often in the Jukskei and other streams that drain urban/developed areas, giving rise to a "spiky" hydrograph with frequencies in the daily range. Assuming that: a) to a large extent the flux of toxicants is controlled by wash-off and dilution and b) the water concentration is controlled by adsorption onto waterborne solids, the observed concentration would likely be flow-related.)

Establishing the minimum frequency: Entropy approach

The hypothesis testing methodology above is based on a counting statistics (i.e. the statistics applicable to large numbers of (usually) independent observations). An entirely different approach would be to make use of information measures. The rationale for using information measures stems from the observation that one of the most significant uses of the NTMP is status assessment. Thus the status can be said to be the information contained in the concentration time series. Status is expressed in terms of a category or class. One can now argue that concentration variability does not necessarily imply class variability. Depending on the classification criteria, all or most of the variability may be lost in classification. So, while there may be significant variability in the concentration time series, there would often be less variability in the class time series. Based on the observed/ inferred distribution of results, it may be possible to assess the class distribution and based on that a class "entropy" could be calculated[2].

Entropy as used here as a measure of uncertainty. The first three steps of the HT methodology above is followed. In the 4th step the entropy is calculated. The question now is: how many samples are needed to get the same level of certainty (entropy) as the highest frequency can give you.

While this approach would be less data-intensive than the HT approach, it still requires sampling at high frequencies to establish a "baseline pattern" which serves as

[2] A concentration time series $Y=\{y_1, y_2, \ldots y_n\}$ can be mapped to a class time series $X=\{x_1, x_2, \ldots x_n\}$ where each x can be one of a limited number (say k) of classes, i.e. $x_i=\{c_1, c_2, \ldots c_k\}$ and from this one can estimate the probability that class c_j will be observed: $P_j = P(c_j)$. The class entropy $S = -\sum_1^k P_j \cdot \ln(P_j)$. This is the Shannon entropy, which is related to the Boltzman entropy in thermodynamics and is a measure of uncertainty.

reference to compare the class entropy. In addition, this approach requires that numerical criteria exist to classify an analytical result. With the exception of DDT, none of the constituents have formal criteria and there are too few DDT data above the detection limit to be used.

None of the data at individual stations are currently sufficient to determine the optimal sampling frequency with either of the approaches. On the pooled data from all the sites, dimethyl-phthalate and simazine are the only constituents that can be used. In both cases a lognormal distribution appears to fit the data reasonably well (Figures 6 and 7)

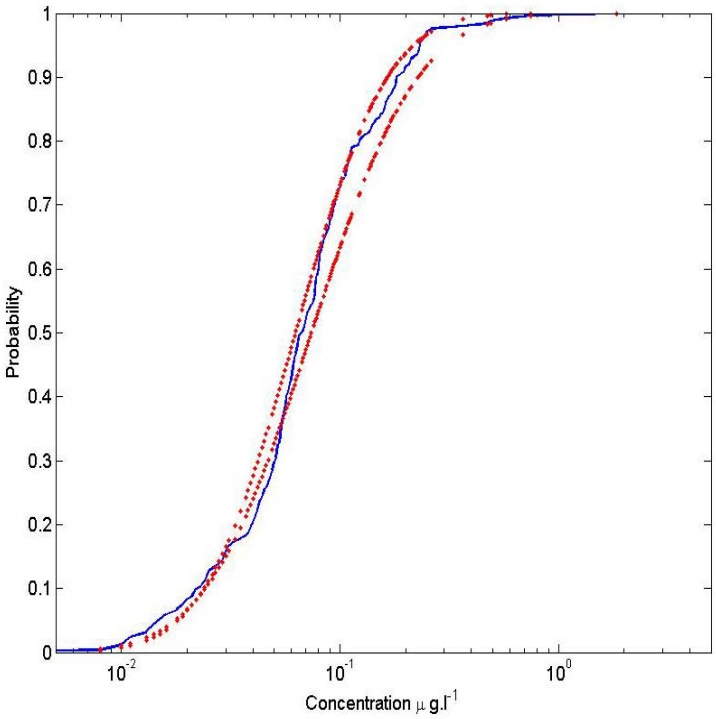

Figure 6. Empirical cumulative distribution for dimethyl-phthalate (solid line) and the 95% confidence interval for a lognormal distribution with μ=-2.687 and σ=0.8123

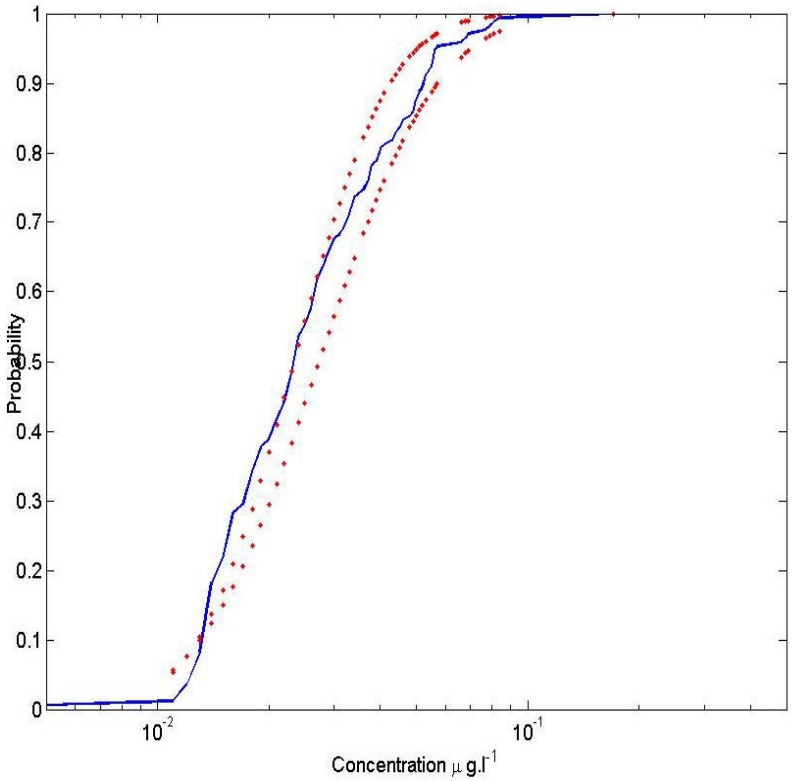

Figure 7. Empirical distribution function on the pooled simazine data (solid line) and the 95% confidence interval for a lognormal distribution function with μ=-3.679 and σ=0.5186

Task 3 Sample Analysis

The standard laboratory protocols of the Directorate: Resource Quality Services' laboratories were followed in both chemical and toxicity analyses (DWAF, 2008). The chemical analyses entailed gas chromatography with mass spectrometric detection/ identification.

The results are summarised in Table 3.

Table 3. Summarized analytical results for pooled data from the pilot phase. The POPs targeted for immediate action by the Stockholm convention are highlighted in grey.

Analysis	# records	% not detected	5th	50th	95th
Acenaphene (µg/l)	199	88	<dl	<dl	0.013
Aldrin (µg/l)	34	94	<dl	<dl	0.016
Arochlor (as 1254) (µg/l)	197	95	<dl	<dl	0.002
Atrazine (µg/l)	305	17	<dl	0.057	0.230
Benzo(a)pyrene (µg/l)	196	97	<dl	<dl	<dl
BHC α- (µg/l)	291	48	<dl	0.024	0.830
BHC β- (µg/l)	256	48	<dl	0.011	0.779
BHC δ- (µg/l)	232	74	<dl	<dl	0.150
BHC γ- (Lindane) (µg/l)	280	45	<dl	0.012	0.105
Biphenyl 2,3,4,5-tetrachloro-4-hydroxy- (µg/l)	26	100	<dl	<dl	<dl
Biphenyl 2,5-dichloro-4-hydroxy- (µg/l)	26	96	<dl	<dl	0.007
Biphenyl, 2,4,6-trichloro-4-hydroxy- (µg/l)	26	100	<dl	<dl	<dl
Biphenyl, 2,5-dichloro-3-hydroxy- (µg/l)	27	93	<dl	<dl	0.020
Chlordane *cis*- (alpha) (µg/l)	36	92	<dl	<dl	0.005
Chlordane *trans*- (gamma) (µg/l)	34	97	<dl	<dl	<dl

Analysis	# records	% not detected	5th	50th	95th
Danio rerio embryos % mortality	103	92	<dl	<dl	35.0
Daphnia pulex % growth, stimulation	35	37	<dl	24.3	46.6
Daphnia pulex % mortality	379	76	<dl	<dl	100
Daphnia pulex % reproduction	21	57	-550	-88	59.8
Daphnia pulex % reproduction, mortality	188	46	<dl	35.0	100.0
DDD, 4,4- (µg/l)	57	42	<dl	0.007	0.030
DDE 4,4- (µg/l)	69	51	<dl	<dl	0.014
DDT 4,4- (µg/l)	223	94	<dl	<dl	0.018
Dibenzofuran (µg/l)	304	46	0.005	0.012	0.065
Dichlorvos (µg/l)	222	97	<dl	<dl	<dl
Dieldrin (µg/l)	35	86	<dl	<dl	0.076
Dimethoate (µg/l)	219	93	<dl	<dl	0.029
Endosulfan sulfate (µg/l)	36	94	<dl	<dl	0.006
Endosulfan-a (µg/l)	37	84	<dl	<dl	0.014
Endosulfan-b (µg/l)	47	66	<dl	<dl	0.044
Endrin (µg/l)	34	97	<dl	<dl	<dl
Fluoranthene (µg/l)	283	30	0.002	0.010	0.049
Heptachlor (µg/l)	35	91	<dl	<dl	0.013
Heptachlor epoxide (µg/l)	33	91	<dl	<dl	0.002
Hexachlorobenzene (µg/l)	56	93	<dl	<dl	0.002
MCPA (metaxon) (µg/l)	187	100	<dl	<dl	<dl
Mirex (µg/l)	36	89	<dl	<dl	0.017
Monocrotophos (µg/l)	34	100	<dl	<dl	0.001
Naphtalene (µg/l)	291	34	<dl	0.013	0.353
Nonyl phenol (µg/l)	187	99	<dl	<dl	<dl
Phenanthrene (µg/l)	288	41	0.005	0.014	0.055
Phenoxy acetic acid, 2,4-dichloro- (µg/l)	187	100	<dl	<dl	<dl
Phtalate dimethyl- (µg/l)	264	47	<dl	0.007	0.119
Phthalate di-*n*-butyl- (µg/l)	292	10	0.011	0.244	1.778
Phtalate, butylbenzyl- (µg/l)	203	72	<dl	<dl	0.254
Phthalate diethyl- (µg/l)	292	22	0.012	0.061	0.307
Phthalate di-*n*-octyl- (µg/l)	196	90	<dl	<dl	0.370
Phthalate, di-*n*-hexyl- (µg/l)	197	84	<dl	<dl	0.025
Poecilia reticulata , % mortality	285	95	<dl	<dl	30.0
Selenastrum capricornutum, % stimulation	371	6	<dl	69.8	140
Simazine (µg/l)	151	4	<dl	0.135	1.530
Terbuthylazine (µg/l)	86	12	<dl	0.065	0.702
Toxaphene (µg/l)	27	100	<dl	<dl	<dl
Vibrio fischeri, % stimulation	382	12	<dl	39.1	59.5

On 3 February 2007 some water and sediment samples were collected for dioxin analysis. These were sent to the company Ökometric GmbH at the Bayreuth Institute for Environmental Research, Germany and analysed on 14 March 2007. The sampling sites and analytical results are shown in Table 4.

Regular statistical distributions were fitted to the pooled data in order to facilitate Monte Carlo methods for the estimation of sampling frequencies. Typical results are shown in Figures 8 to 12 below. The results show that with few exceptions (the triazenes and some of the phthalates) regular distributions (green and red lines) tend not to fit the empirical distribution (upper, stepped blue line) sufficiently. To a large extent this might be due to the low incidence of the pesticides above their detection limits in the data set.

Table 4. Dioxin and PCB analyses near NTMP sites

Sample	*Dioxins[3] ng/l or ng/kg (WHO TEQ)*	*PCB[4] µg/l or µg/kg dry weight (for sediment)*					
		28	**52**	**101**	**138**	**153**	**180**
Klip River at NTMP site (sediment)	0.8	0.51	0.31	0.62	1.19	1.19	0.87
Klip River downstream of NTMP site (sediment)	0.4	0.24	0.12	0.21	0.33	0.31	0.19
Kleinspruit (water)	<0.017	0.04	<0.01	<0.01	<0.01	<0.01	<0.01
Kleinspruit (sediment)	<0.1	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Waterval river at Roodebank (water)	<0.017	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Waterval river at Roodebank (sediment)	4.6	0.21	<0.10	0.12	0.22	0.19	<0.10
Jagspruit (water)	<0.017	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
Jagspruit (sediment)	0.1	0.15	<0.10	<0.10	0.12	0.10	<0.10

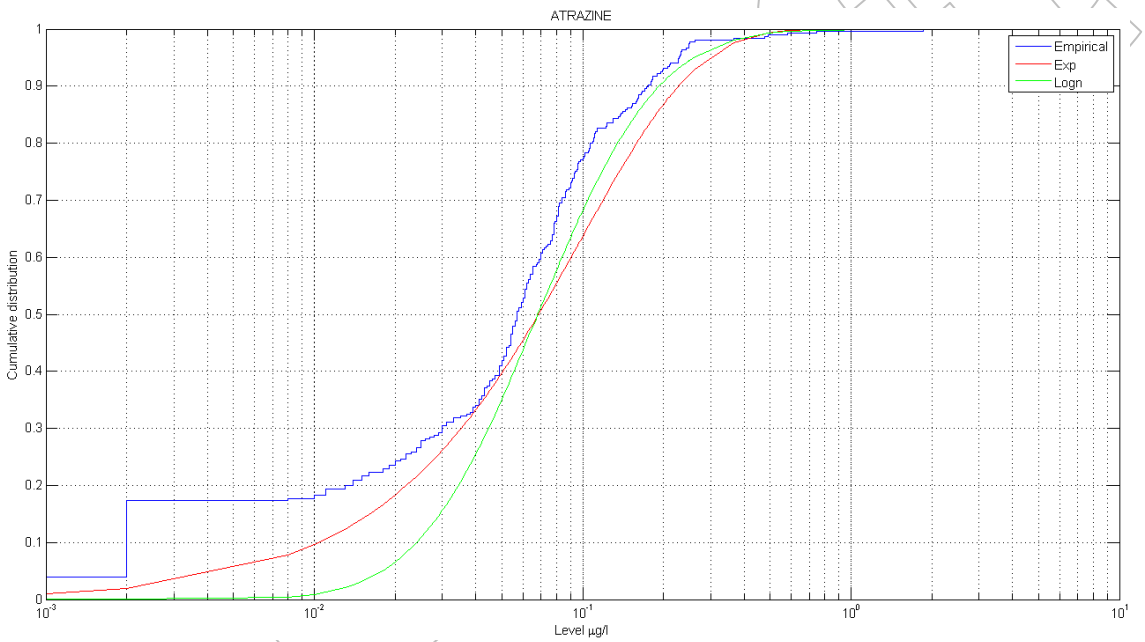

Figure 8. Atrazine concentration distribution on pooled data.

[3] Dioxins here refer to the sum of various polychlorinated-dibenzodioxins and -dibenzofurans expressed as 2,3,7,8-tetrachloro-dibenzodioxin. These might consist of mixtures of tetra-, penta-, hexa-, hepta- and octa-chlorinated isomers. Except for the Waterval River sediment, OCDD is the dominant isomer. The water River sediment contains a range of isomers.

[4] PCB: polychlorinated biphenyl (various congeners). The levels are expressed as toxicity equivalents of 2-,3-,7-,8-terachloro dibenzodioxin (the most poetent of the isomers) according to the World health Organization (WHO) protocol.

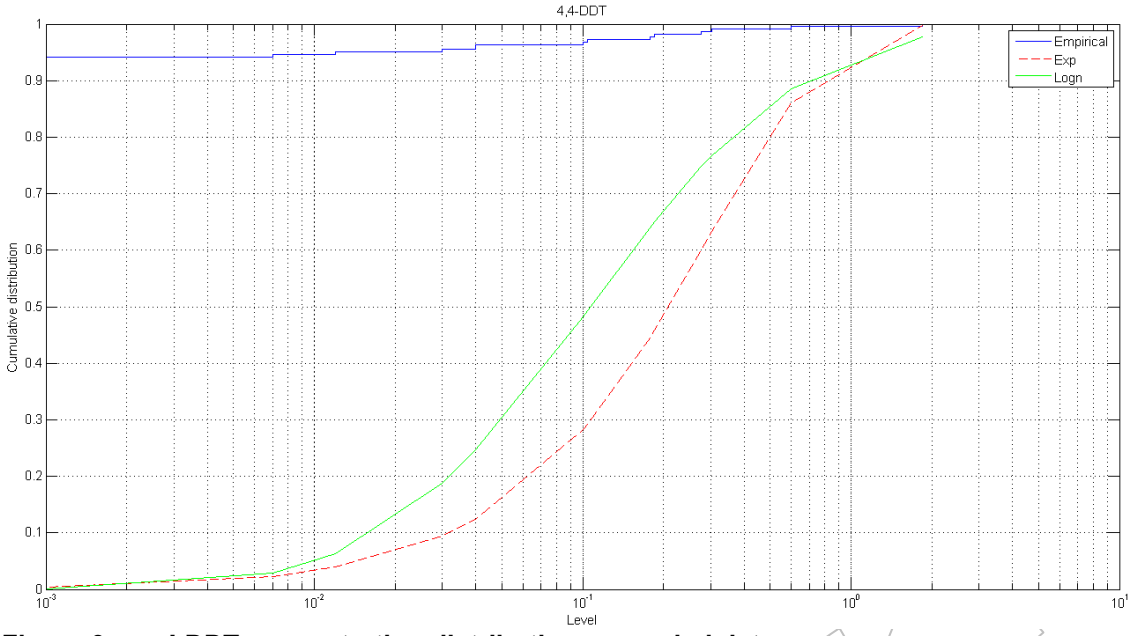

Figure 9. p-,p'-DDT concentration distribution on pooled data

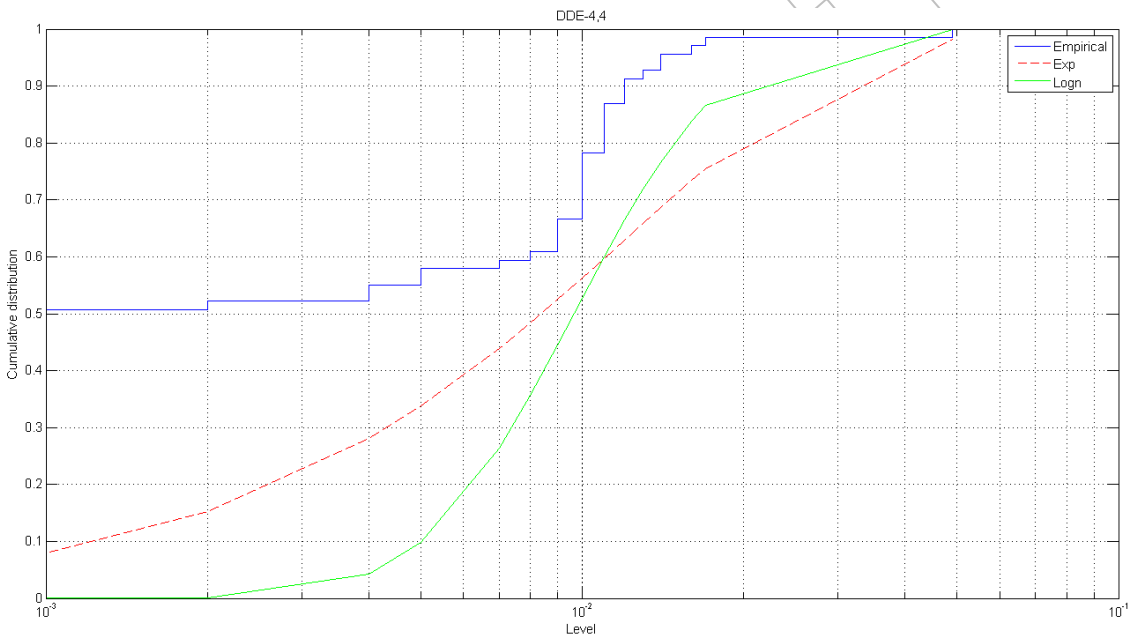

Figure 10. p-,p'-DDE concentration distribution on pooled data.

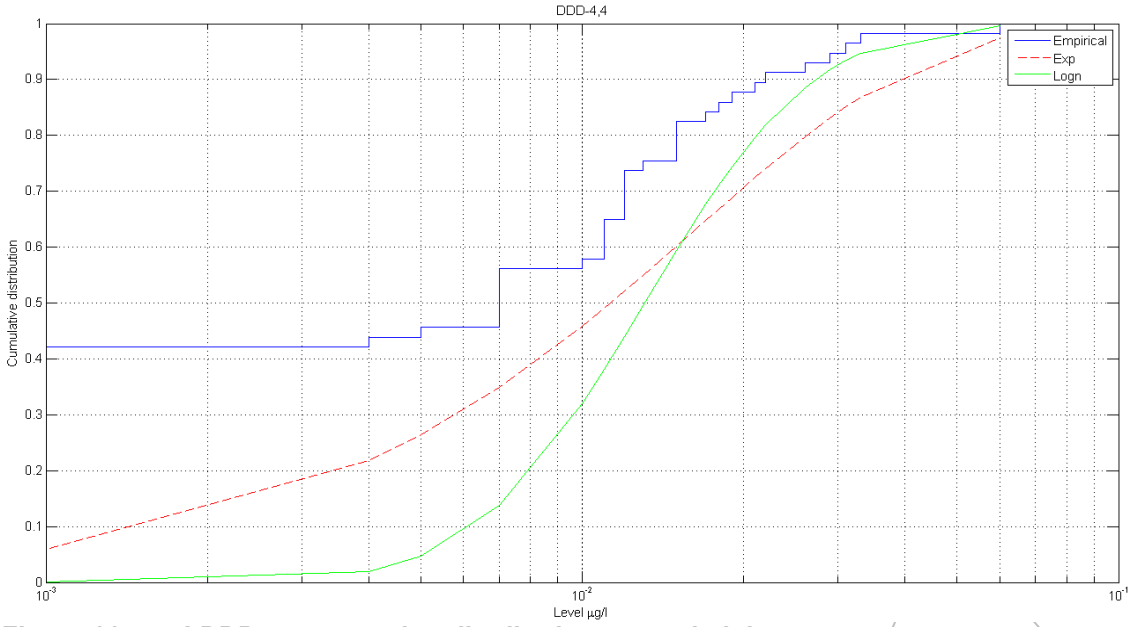

Figure 11. p-,p'-DDD concentration distribution on pooled data.

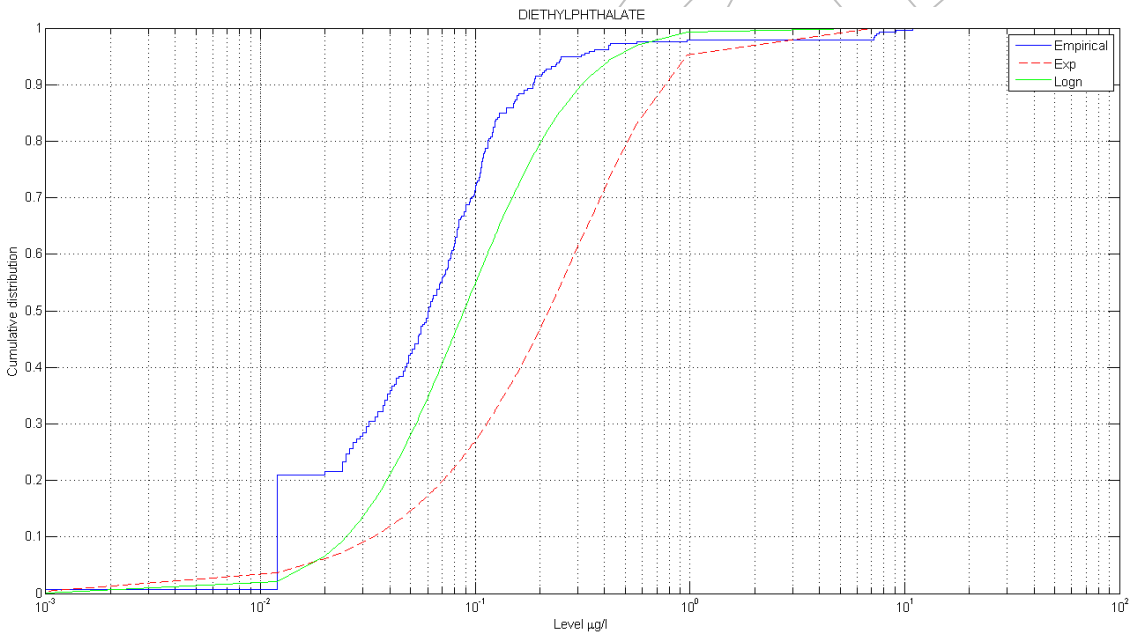

Figure 12. Diethyl-phthalate concentration distribution on pooled data

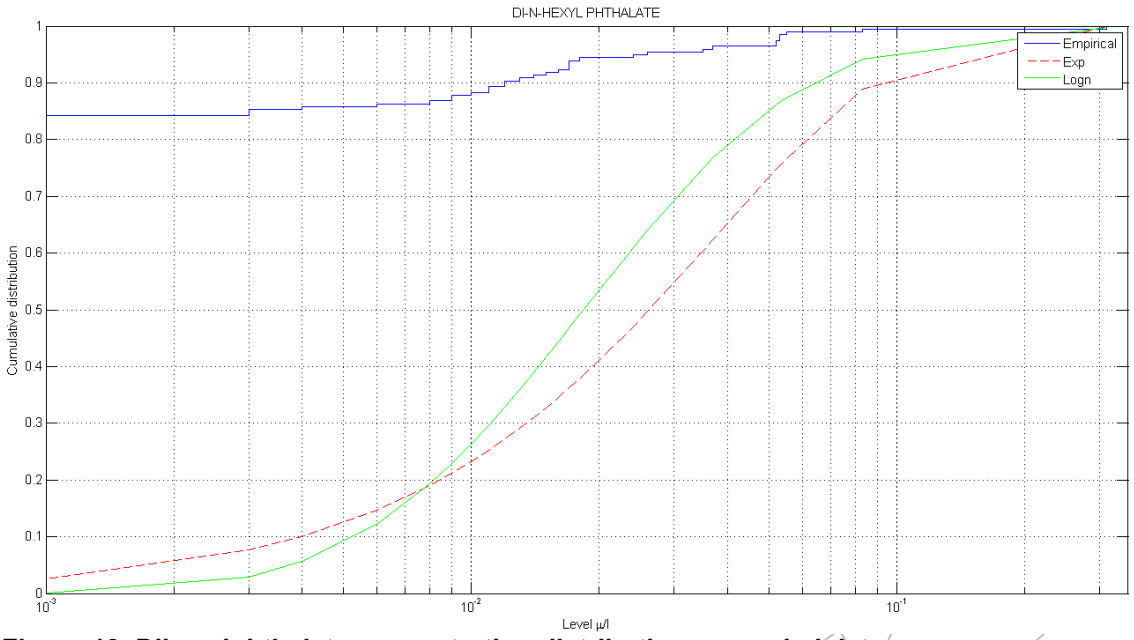

Figure 13. Dihexylphthalate concentration distribution on pooled data.

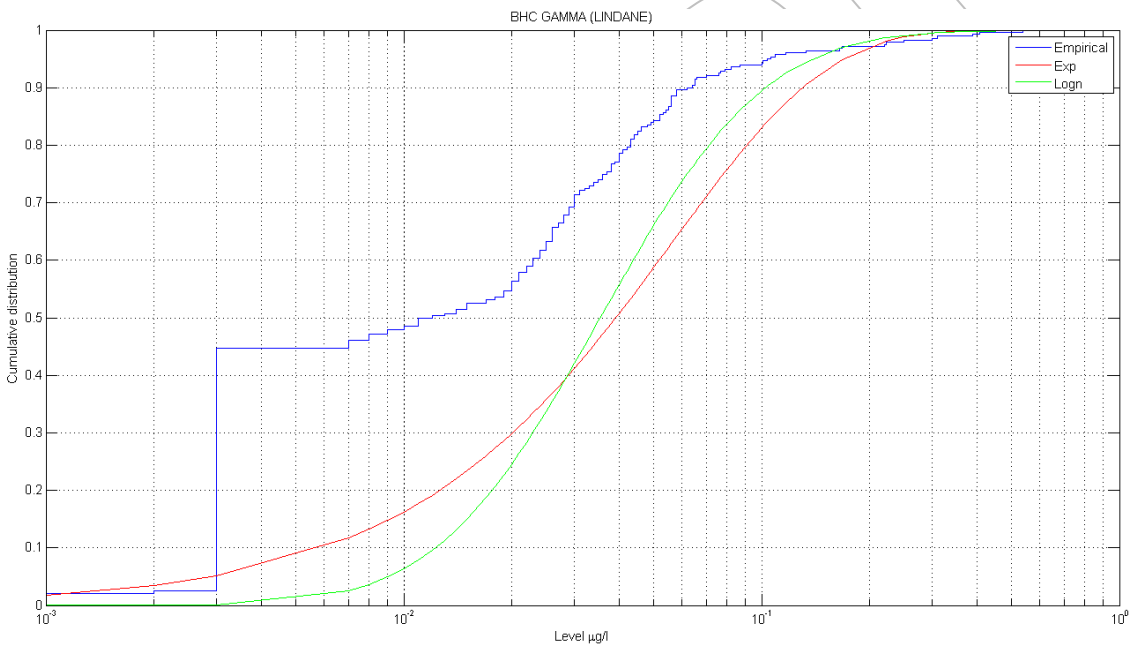

Figure 14. Lindane concentration distribution on pooled data.

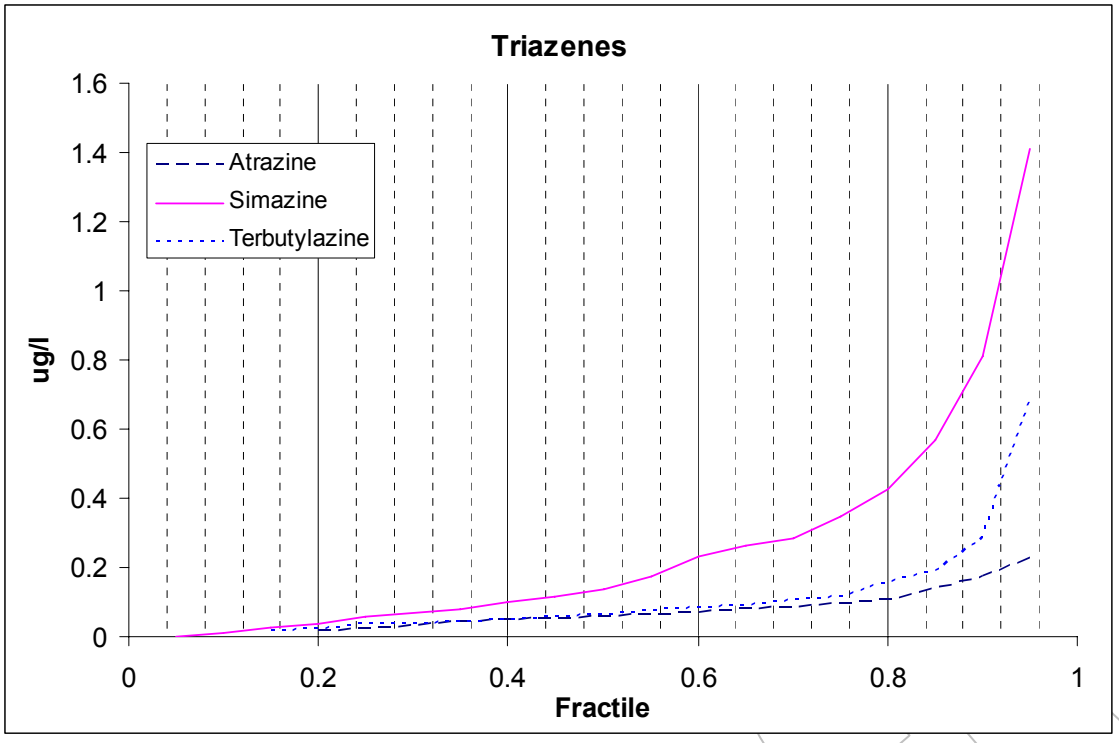

Figure 15. Pooled triazene herbicide concentration fractiles.

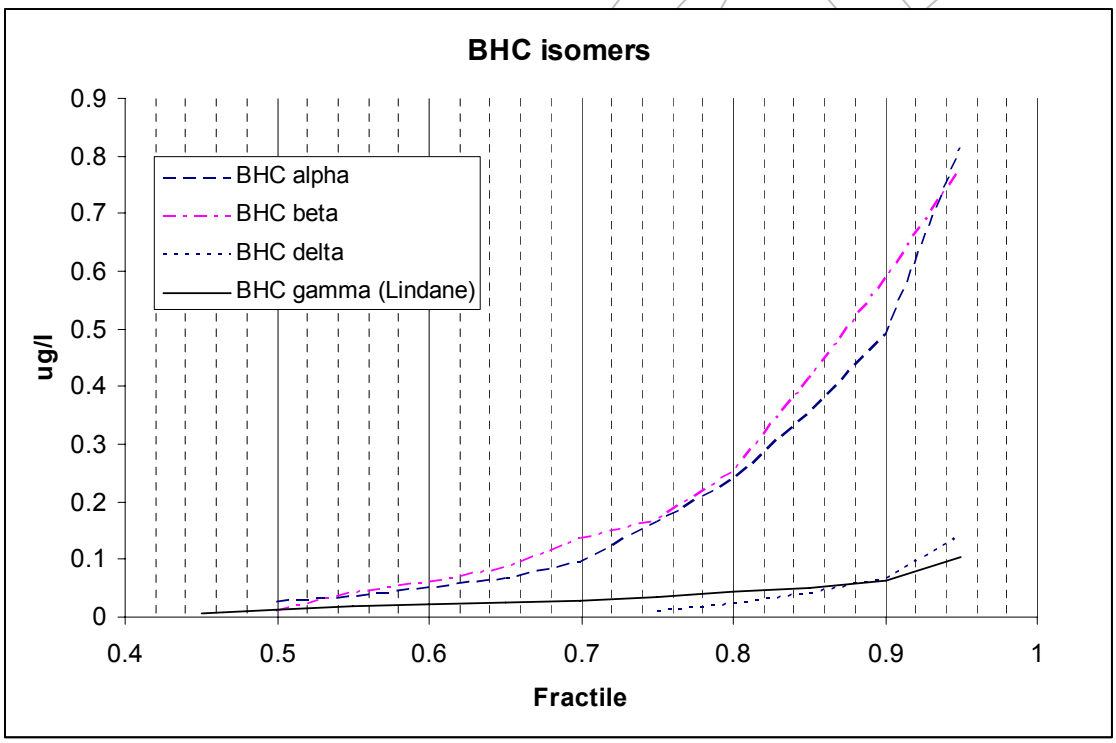

Figure 16. Pooled BHC concentration fractiles

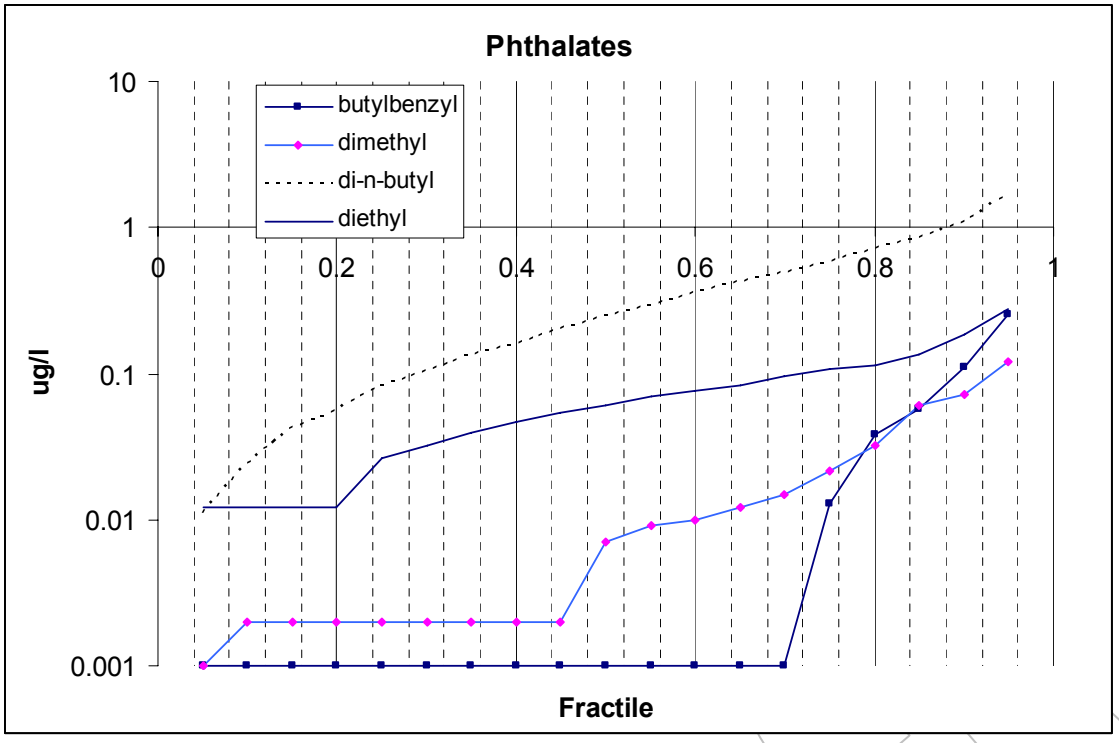

Figure 17. Pooled phthalate concentration fractiles

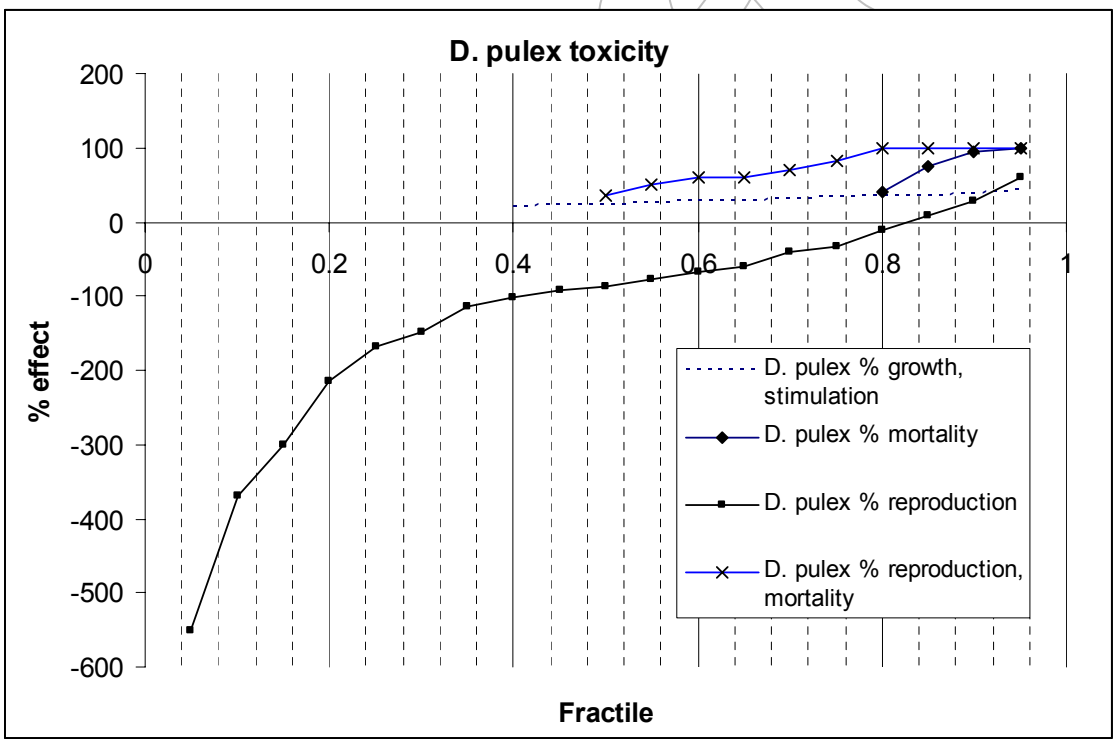

Figure 18. Pooled Daphnia pulex toxicity fractiles.

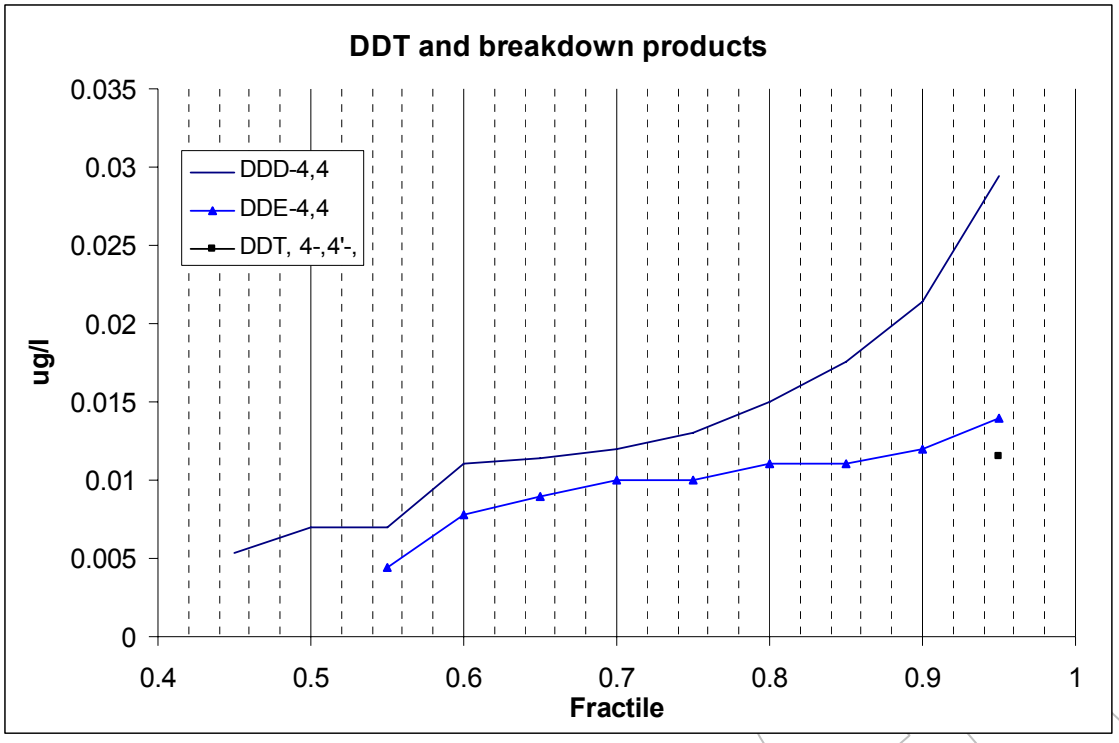

Figure 19. Fractile plots of pooled concentration of DDT and its breakdown products.

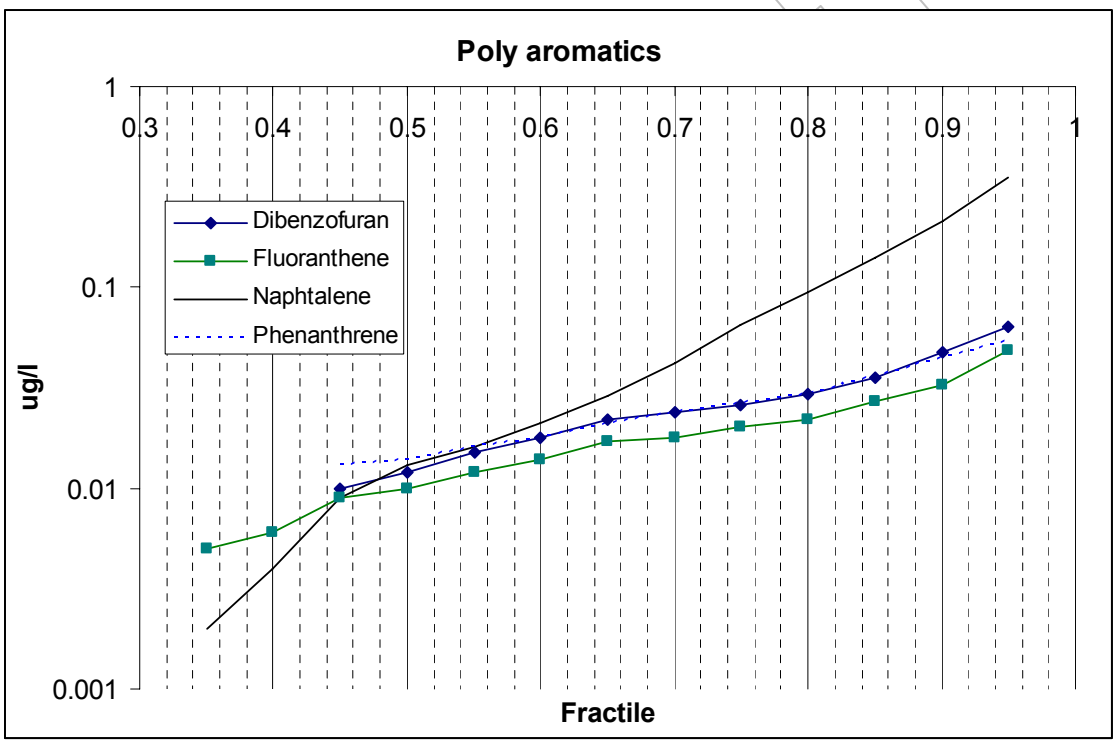

Figure 20. Pooled poly-aromatic concentration fractiles.

The data presented here supports the intuitive notion that if a constituent is seldom detected, let alone quantified, then it is practically impossible to optimize the sampling frequency based on the observed data. As it stands, it is not possible to conclude that any lower than weekly sampling will suffice for trace constituents.

For the toxicological properties the picture is qualitatively the same. However, if measures could be found that is more prone to a graded response (such as in the case of biomarkers), the situation for toxicological assessment could be markedly improved even though the interpretation may be substantially more difficult

- Inclusion of other toxicants (e.g. further pesticides and trace metals)
- Are the proposed toxicity tests: hER and *B. rerio* and chronic *D. pulex* appropriate tests to be phased in?

Task 4 Data management and Storage

The quality controlled analytical data were entered by hand and stored in the Water Management System (WMS) database maintained by the directorate: Resource Quality Services of the Department of Water Affairs and Forestry at Roodeplaat Dam near Pretoria.

Task 5 Information Generation and Dissemination

Inspection of the analytical results (Appendix 1) and the fractile plots on pooled data (Figures 15 to 20) show that:

- The bulk of the chemical constituents analysed for are below the current method detection limit.
- The bulk of the toxicity assessments show low (often no) response or (in the case of algal test) stimulation of growth rather than inhibition
- The worst site is at Marlboro on the Jukskei River.
- The most commonly occurring chemicals in the group analyzed for are the phthalates and the triazine group of herbicides.

For a global picture the data from all the sites were pooled as a screening step. This produces a picture that is biased toward the condition in the Jukskei River from which about five times as many samples are represented as compared to the Middle Vaal. Nevertheless, those variables for which no problem can be detected on the pooled data (using medians and maxima) probably do not need further analysis.

The assessment of the analytical results requires guideline values. The interim guideline values were developed in Phase 1 of the NTMP and the values for the aquatic ecosystem are shown in Table 5.

Table 5. Interim guideline values for unspecified end-points for aquatic ecosystem classification. Targeted compound highlighted in grey.

Toxicant	***Class boundaries (µg/l)***			
	Natural	**Good**	**Fair**	**Poor**
Aldrin	< 0.04	0.04 – 0.15	0.15 – 0.29	> 0.29
Atrazine	< 0.14	0.14 – 1.3	1.3 – 11.65	> 11.65
Chlordane	< 0.03	0.03 – 0.08	0.08 – 0.14	> 0.14
DDT	< 0.006	0.006 – 0.01	0.01 – 0.03	> 0.03
Dieldrin	< 0.11	0.11 – 0.24	0.24 – 0.39	> 0.39
Endosulfan (α+β)	< 0.01	0.01 – 0.07	0.07– 0.57	> 0.57
Endrin	< 0.03	0.03 – 0.05	0.05 – 0.08	> 0.08
Heptachlor	< 0.02	0.02 – 0.16	0.16 – 0.39	> 0.39
Hexachlorobenzene	< 0.14	0.14 – 0.31	0.31 – 0.53	> 0.53
Lindane	< 0.24	0.24 – 0.51	0.51 – 0.83	> 0.83
Mirex				>0.04
Monocrotophos	< 0.002	0.002 – 0.05	0.05 – 1.68	> 1.68
Simazine	< 0.7	0.7 – 6.67	6.67 – 63.7	> 63.7
Toxaphene	< 0.06	0.06 – 0.17	0.17 – 0.28	> 0.52

Applying the guideline values in Table 5 to the results in Table 3 yields the assessment in Table 6.

Table 6. Ecological assessment for unspecified end-points of pooled water column analytical data. Targeted compound highlighted in grey.

Analysis	*Median (µg/l)*	*95th (µg/l)*	*Category*
Aldrin	<0.001	0.016	**Natural**
Atrazine	0.057	0.230	**Good**
Lindane	0.012	0.105	**Natural**
Chlordane	<0.001	<0.001	**Natural**
DDT, 4,4-	<0.001l	0.018	**Fair**
Dieldrin	<0.001	0.076	**Natural**
Endosulfan	<0.002	0.058	**Fair**
Endrin	<0.001	<0.001	**Natural**
Heptachlor	<0.001	0.013	**Natural**
Hexachlorobenzene	<0.001	0.002	**Natural**
Mirex	<0.001	0.017	**Natural**
Monocrotophos	<0.001	0.001	**Natural**
Simazine	0.135	1.530	**Good**
Toxaphene	<0.001	<0.001	**Natural**

Table 7. Assessment of individual sites for ecological impact of pesticides

Toxicant	*Marlboro*	*Midrand*	*N14*	*Kleinspruit*	*Klip River*	*Jagspruit*
Aldrin	Fair	Natural	Natural	Natural	Natural	Natural
Atrazine	Natural	Good	Natural	Good	Natural	Natural
Chlordane	Natural	Natural	Natural	Natural	Natural	Natural
DDT	Natural	Natural	Fair	Poor	Fair	Fair
Dieldrin	Natural	Natural	Poor	Natural	Natural	Natural
Endosulfan (α+β)	Natural	Natural	Good	Natural	Natural	Natural
Endrin	Natural	Natural	Natural	Natural	Natural	Natural
Heptachlor	Poor	Natural	Natural	Natural	Natural	Natural
Hexachlorobenzene	Natural	Natural	Natural	Natural	Natural	Natural
Lindane	Natural	Natural	Natural	Natural	Natural	Natural
Mirex	Natural	Natural	Poor	Natural	Natural	Natural
Monocrotophos	Natural	Natural	Natural	Natural	Natural	Natural
Simazine	Natural	Natural	Natural	Natural	Natural	Natural
Toxaphene	Natural	Natural	Natural	Natural	Natural	Natural

Table 8. Assessment of pooled toxicity data from water column samples

Analysis	*% samples showing effect*	*5th*	*50th*	*95th*	*Assessment*
Danio rerio embryos and larvae % mortality	8	0.008	0.008	0.028	Natural
Daphnia pulex % growth, stimulation	63*	0.063	0.153	0.294	Natural
Daphnia pulex % mortality	24	0.024	0.024	0.24	Good
Daphnia pulex % reproduction	43*	-2.37#	-0.378	0.257	Fair
Daphnia pulex % reproduction, mortality	54*	0.054	0.189	0.54	Poor
Poecilia reticulata , % mortality	5	0.005	0.005	0.015	Natural
Selenastrum capricornutum, % stimulation	94	0.094	0.656	1.32	Natural
Vibrio fischeri, % stimulation	88	0.088	0.344	0.524	Natural

*Possibly not significant due to limited samples

Negative values indicate stimulation

DWAF(2005) suggests a simple assessment guide based on the occurrence of lethality or the absence of any (including chronic) effects. In this context only the *Daphnia pulex* results can be used since it is the only organisms for which both lethality and sub-lethal endpoints is known. This was conceptually derived from a procedure (Jooste and Rossouw, 2002) used to derive numerical water quality guidance for the determination of the Ecological Water Quality Reserve, based on data of LC50 and NOEC/LOEC values. In that case the exact level of effect on exposure was unknown. In the data recorded for this project the maximal mortality is known. This provides an indication of the "intensity" of the effect. One can now calculate a "risk-like" number combining the probability (frequency) of observing an effect in a population and the probability (frequency) of its occurrence in the water resource. The probability of two independent events (i.e. the occurrence of stress and the level of stress) is calculated as the product of the individual probabilities. This number is reported in Table 8.

The toxicity results in Table 8 shows that while short term mortality is at a low level, in the longer exposure scenarios mortality is more common, it is not entirely clear that this is due to toxicants in the water sample or whether it is due to stress because of general habitat stress due to differences in the "non-toxic" water quality template, such as turbidity and temperature. Table 9 provides an assessment of the toxicity data on a site-by-site basis.

All sites evidence some toxicity. Comparing Tables 7 and 9 it is apparent that the toxicity and chemical assessments are not in agreement. To be more precise there is no simple logic to combining the analytical results to correspond to toxicity results. The discussion in the record of decision on the NTMP (DWAF, 2005) stated that the chemical and toxicological analyses are complementary and not meant to be supporting. These data bear out this view.

Two issues arise from these data: 1) are the toxicity tests sufficiently sensitive to act as indicators of biotic effects in the rivers? and 2) is the methodology used in deriving the assessment criteria perhaps overly conservative? From the low occurrence of responses one can conclude that the water tested is largely non-toxic. This is borne out by the observation that live and apparently healthy organisms are caught while sampling the river water. At the same time one cannot make any pronouncement on the state of the water with respect to more sensitive organisms than those used in the test. The chemical assessments are based ultimately on statistical manipulations that may produce benchmarks relating to species that have not been tested. In this way the chemical assessments are complimentary to the toxicity assessments. Despite this, the large number of toxicity non-detects causes statistical problems. It would therefore be ideal to have a toxicity test that produce a continuum of response at lower concentration levels that those representing NOECs in the current suite of toxicity tests, in conjunction with the necessary assessment criteria to be used in the interpretation of these results.

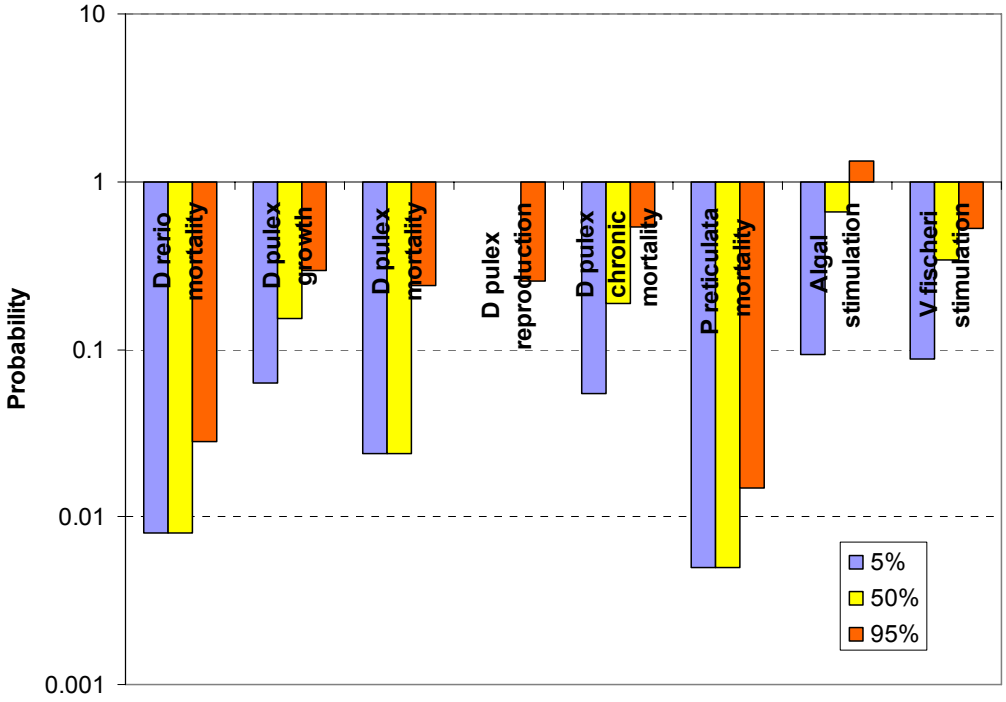

Figure 21. Individual risk from pooled data for various toxicity test species. Values > 1 indicate stimulation

Figure 21 shows that the highest probability of response occurs in the lower organisms or those that depend on sub-cellular responses such as *V. fischeri*. This suggests that *V. fischeri* might be a useful surveillance test since it appears to be the most conservative across all percentiles of effect while at the same time not suffering the statistical inconvenience of stimulation effects. This is understandable in that the mechanism of test response relates to enzymatic inhibition, which emphasizes the potential usefulness of sub-organismal or even sub-cellular methods in environmental toxicity assessment. This issue is taken up under Task 7. It is clear though that the interpretation of the toxicity results needs careful attention.

An issue that crops up in many chemical assessments relates to dealing with mixtures of chemicals. This is currently a topic of investigation internationally as shown by the attention it receives from regulatory authorities (e.g. USEPA, 2000). Locally it may well form part of the work on the review of the 1996 South African Water Quality Guidelines (Jooste 2007).

Table 9. Assessment of the toxicological data per site.

Site	*50th*	*95th*
Marlboro	Good	Fair
Midrand	Good	Good
N14	Good	Poor
Kleinspruit	Good	Good
Klipspruit	Good	Good
Jagspruit	Good	Good

Task 6: Capacity building-plan and cost estimate of full-scale implementation of the NTMP.

Cost

The cost for phase 3 of the project to date can be divided into costs for a) sampling and b) analyses. In this phase the sampling effort was combined with other programmes in order to save costs. For this reason it is not always clear what the actual expenditure on this phase was. Estimates are based on calculation by interpolation. These estimates are shown in Table 10.

Table 10. Expediture estimates for pilot phase of the NTMP

Route	***# Samples***			***Sampling***			***Sampling Cost***			***Analytical cost***
	Sampled	**Org**	**Tox**	**km**	**h**	**R/h**	**Trav**	**Time**	**Total**	
Jukskei	90	88	70	18900	450	250	44 415	R112 500	R156 915	R 517 070
Middle Vaal	41	35	23	26240	410	350	61 664	R143 500	R205 164	R 185 109
Dioxin	8	-	-	-	-	-	-	-	-	R4 240
Totals									R362 079	R 706 418
Grand total									R 1 068 497	

In interpreting the cost estimate in Table 7, it should be borne in mind that there were significant analytical development and method establishment costs involved in the both the organic and toxicity laboratories that are not reflected here. These costs are difficult to quantify as they are of often part of the operational overheads of these laboratories as well as capacity development.

The sampling time and analytical cost are absorbed in the RQS budget, so that the only direct expenditure was travel cost which amounts to R107 000. It is noteworthy that in the given sampling scenario, the sampling cost is still only about a third of the total cost to the fiscus. This means that investment in research and development to improve analytical efficiency would be beneficial.

If the sampling regime had been adhered to strictly the total cost would have been R3.3M with a direct expenditure of about R0.8M. The saving was largely brought about by consolidating the sampling effort between programmes.

Capacity development

One of the most critical needs that have surfaced through phase 3 of the NTMP is the need for establishing a skilled organic analytical work force in an enabling environment. The problems centred around:

a) Skills-related issues such as adapting methods to the physical characteristics of the sample, the insightful operation and interpretation of instrument output, and varying levels of interest and dedication.
b) Laboratory environmental issues such as instrument breakages, difficulty in obtaining appropriate reference chemicals, budgetary limitations etc.

Organic chemical analysis is recognized to be a difficult aspect of chemical analysis, requiring a high level of appropriate skills. These skills include: 1) a high level of academic training in principles of organic and physical chemistry, 2) insight into organic environmental chemistry, 3) high level of operational skill and insight in the principles of operation of the instrumentation used, 4) logical thinking ability and 5) an enabling environment which includes the ability to remain up-to-date with development in the discipline.

From the above it is clear that the appropriate skills mix is difficult to find. In a market where chemists are scarce, organic analysts are even more so. A suitable analytical laboratory should always have an at least one experienced organic analyst to mentor and guide the work. The ideal staffing of an organic laboratory for the NTMP is shown in Table 11.

Table 11. Ideal staffing for an organic analytical laboratory for the NTMP

Description	*Typical qualifications*	*Function*
1 Specialist Scientist	At least M.Sc (or equivalent) in discipline related to Organic analysis and min 5 yrs appropriate experience.	To provide ongoing technical guidance to lab staff, initiate innovations in analytical methodology, instrumentation, sampling methods, etc. Keeping abreast of technology/ developments in analysis of water, sediments and aquatic biota for organic pollutants. Assist with auditing, QC and QA procedures, assist with in-house training. Give presentations on analysis of organic toxicants as required.
1 Chief Technician	B.Sc (Hons) in chemistry or equivalent with min 3 yrs appropriate experience.	Supervise day-to-day running of the lab, scheduling (production, training, etc), admin, QC, maintenance issues, internal auditing, inventory control, etc. Must have hands-on involvement with analyses in order to perform these functions.
2 Senior Technicians	BSc or Nat. Dip. in Analytical Chemistry with min 2 yrs appropriate experience	To perform analyses of routine and *ad hoc* (special) samples under supervision of the Chief Tech and/or Hydrologist.
1 Auxilliary Technician	Matriculation with chemistry and physics. Min. 2 yrs lab experience	To perform specialized cleaning of glassware using procedures appropriate for organic residue analysis, assist with inventory control, sample management and primary preparation, general tidiness and temperature recordings, etc.

It might be useful to investigate an infrastuctural model where the scarce human resources in organic analysis in the country can be pooled, for example in a central laboratory where the needs of various institutions can be catered for. This is clearly a matter that needs to be addressed at a top management level among various interested and affected agencies.

Task 7: Development work

The toxicity methodology used in this phase conformed to what was commonly available during phases 1 and 2 of the NTMP. From the results above it is clear that more often than not the water samples gave no result. While this observation has value in itself, it does cause some statistical difficulties. If the bulk of the responses are

below the detection limit, then it becomes very difficult (or impossible) to characterise the occurrence of toxicity. At an ethical level the question can be raised whether the selected tests are in fact valid for drawing conclusions about anything other than mortality.

Biomarkers

Appendix 3 contains an assessment of the toxicity testing needs of the NTMP. The conclusion is that biomarkers could successfully be used in the NTMP. These include:

- Activity of various esterases and specifically acetylcholine esterase (AChE).
- Mixed function oxygenases (MFOs) activity such as EROD coupled with AChE.
- Cellular Energy Allocation(CEA) which is a good general stress biomarker especially when coupled with another specific biomarker such as AChE and EROD. With the use of multivariate statistics there is a possibility to identify which responses correlate with energy expenditure and such information is helpful in identifying priority problem toxicants.
- Glutathion-*S*-transferase (GST), which detects exposure to toxicants but may also indicate the organism's physiological response to minimize toxicity.
- The assessment of metallothionein activity for metal exposure

The application of these tests needs to be investigated.

Trace elements

During this phase trace metal were not included in the study. The reasons were that: a) This programme was considered to focus on toxicity (a biotic effect) rather than on toxicants (causes of the effects), and b) none of the metals were included among the 'dirty dozen' of POPs targeted for immediate action by the Stockholm Convention. It has, however, become increasingly clear that for the foreseeable future chemical analysis and biological assays will remain complementary techniques addressing different data user needs. In the light of this, the inclusion of trace element analysis must be investigated. For immediate attention those elements such as cadmium, arsenic, lead and mercury, that are known to have acute effects, should be included. Recent studies in the Western Cape and in Mpumalanga showed a high frequency of pesticide exposure in surface waters. These pesticides are currently not included in the NTMP and might need further attention.

Media to be sampled

At present the NTMP is focussed around the water/biota interaction (Figure 22). During the design phase a deliberate decision was taken not to address bottom sediments in river systems. The settled solid phase is known to be less homogeneous than the overlaying water phase. The reasons for this may relate to the differential settling ability of solids from flowing water due to variability in physical characteristics of the solid phase and these in turn may also result in differing physico-chemical characteristics such as specific adsorption. Capacity to deal with such media complexity is still a limiting factor internationally.

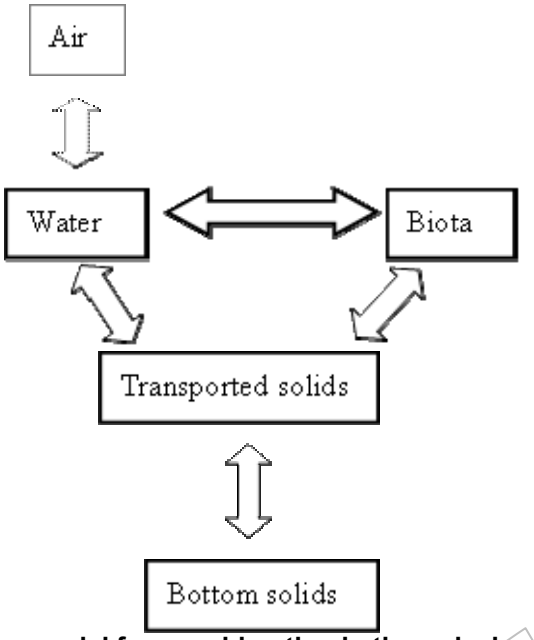

Figure 22. Water resource model for consideration in the redesign of the NTMP

The data collected during Phase 3 of the project yielded unexpected results. Many of the chemicals that were thought to have been present were in fact not found. The results so far were inconclusive as to whether they were absent from the system or whether they were simply adsorbed in the sediment. It is noteworthy that the substances that were found in the water analyses were mostly those with higher water solubility, mostly more polar compounds. Many of the targeted POPs have low water solubility, i.e. a high inclination to partition into the organic solids or simple precipitate out of solution (this latter effect will be exacerbated by increased salinity). In addition the Stockholm convention documentation was quite clear that the largest concentration would be found in biota and sediments.

Ultimately however, the assessment of the status of the national surface water resources and the rivers and streams in particular, does not depend on the water column only. A water constituent or contaminant partitions among various stream compartments in a state of pseudo-equilibrium. In order to assess the true state of the resource this pseudo-equilibrium needs to recognized, since a disruption in one compartment induced changes in other compartments BUT not necessarily linearly and usually not immediately (i.e. both the position of the equilibriums and the rate at which it is reached are generally complex functions of other stream variables, Chapra, 1996). The current monitoring design does not accommodate this.

All of the above argues for including the sediment in the monitoring of toxicity in the resource or possibly the establishment of a separate programme that deals with the finely divided solid phase in river systems. But, there are a number of issues that need attention before this can be done:

Direct assessment of toxicity in sediments

The mineral and other solids in river systems not only play a role in the physical modulation of water column concentrations of chemicals, it is also an important aquatic biotic habitat component in its own right. It is therefore fitting that the quality of this habitat also be assessed. The direct assessment of the toxicity displayed by sediment needs to be measured. This is a fairly new field in aquatic ecotoxicology but some reasonable well established techniques are available and are currently explored by

RQS. These techniques can generally follow one of two approaches: a) separating the interstitial water[5] and using the better established aquatic toxicity techniques or b) using sediment dwelling organisms (i.e. those that spend either a part or all of their life history in sediment) to measure toxicity. Not only do the techniques have to be investigated but also the interpretation of the results in terms of classification and its relationship to water column toxicity needs to be clarified. It appears likely that there no 'off-the-shelf' solutions available, some research and development is indicated.

Chemical analyses of sediments

The chemical analysis of sediments presents various conceptual and technical difficulties. At the conceptual level issues such as what kind of chemical content should be assessed, for example one can distinguish among total content, chemicals that can be desorbed under various conditions such as acidic conditions, alkaline conditions, high or low organic concentration in the overlying water, high or low redox potential in the overlying water or in the sediment itself, the amount immediately available to biota, the amount available to biota in ingestion, etc. Each of these have interpretational domains. Some investigation and possibly research is needed to establish which is to be required to satisfy the needs of the NTMP.

At a technical level it is conceivable that the organic content, mineral composition and physical characteristics of the solids material could have a significant bearing on how the analyses are performed. How these aspects affect the analytical results needs to be clear before sediment data becomes part of a monitoring programme.

Sediment sampling

How sediment is sampled depends very much on exactly how sediment is defined. It is clear that the particle size distribution of solids transported in flowing water systems can span the range from nanoparticles through colloids to pebbles and rocks, depending on the flow energy of the stream. Even in stagnant systems there is often not a clear distinction between what the water column is and what the bottom sediment is, due, in part at least, to electrostatic phenomena. Many of the mechanisms by which chemicals partition themselves between phases are related to the physico-chemical characteristics of the particle surface, implying that they are also dependent on the surface area and mineralogical composition of the particles. Therefore, the deposition of solids in a river system and hence the macro- and micro-sampling site selection as well as the sampling and analytical techniques are likely to be a function of flow and geomorphology of the river system and the mineralogy/chemical composition of the solids. The effective and efficient sampling and analysis of sediment is a matter that needs to be investigated further.

Analysis of dioxins

One of the more troubling aspects of POPs analysis currently is the inability to have analyses of the chlorinated-dibenzodioxins and –dibenzofurans performed locally. They might be fairy ubiquitous in the aquatic environment but the acceptance or rejection of this hypothesis requires a fairly large number of analyses. The establishment of a laboratory to perform these analyses in South Africa needs to be considered as a matter of urgency.

[5] We distinguish between pore water and interstitial water. Nominally, interstitial water refers to that component of sediment that fill gaps between particles and which can be separated by gravitational techniques such as centrifugation. Pore water is that component which is included in the sediment particles (in pores and fissures) and which can generally only be removed by active displacement and/or drying and which is subject to capillary action and diffusion.

Characterising river loads

The frequency of sampling performed in Phase 3 was regulated by cost and facility considerations. As was shown in section on frequency determination above, there is a need to characterise the variability in concentrations of the compounds more carefully. Generally this would require high frequency sampling. High frequency sampling can be prohibitively expensive. An important step in designing a project to estimate the variability in the concentrations of toxicants is to establish whether they are present in the system at any given time. If a particular compound is not found during grab sampling, it simply indicates that it was not present in detectable quantities at that particular instant in time. What is required is a means for "integrating" or summing the concentrations over a period of time.

The use of integrative samplers might therefore merit attention. These devices are usually in some way based on the flow through systems containing some form of selective absorbing material. It is meant to supply some idea of the summed or integrated load of the adsorbed material in the stream. To the extent that these devices are flow-dependent, the integrated load can be partitioned with a knowledge of the hydrograph at the sampling point. This requires some focussed investigative work.

Risk characterization of river systems

It is obvious that analytical-, and to some extent toxicological results by themselves are not useful – they need to be contextualized in some way. The assessment paradigm current during the design of the NTMP centred around hazard, i.e. the potential for observing an adverse effect. The typical assessment scenario comprises taking the analytical results of sample, comparing it to some numerical criterion (such as those found in the South African Water Quality guideline series of 1996) and classify the outcome accordingly. This, however, does not really provide the full answer for two reasons: a) generally the effects that correspond to the various numerical values are immediately apparent and b) the extent to which the sample represented the river system, which is defined in a much larger geographic and even temporal domain represent any particular water user is not apparent. Risk characterization provides a means to address this difficulty.

It is apparent that for different effects and different use scenarios significantly different assessment outcomes are possible. There is currently a review of the South African Water Quality Guidelines series under way which may help to address the differing effects issue. More investigation on how to address the temporal and geographic variability and/or uncertainty is required in order to provide a reasonable and scientifically tenable assessment of the status of a river system. The risk paradigm may help to address this.

Chapter 4 Recommendations

The data collected so far already yields information that was not available before, even though the interpretation of the data is not unequivocal as yet. There is no other data currently collected by DWAF either directly or through other agencies that can yield the same insights into the status of toxicants and toxicity in river systems. Even at a cost of more than R1 000 000, this expenditure is justifiable.

The results in Chapter 3 show that the concentrations of toxicants are generally low in the water column at the selected sampling sites. This might be due to any of the following reasons:

1) There are really very little of the toxicants present in the system,
2) The toxicants are in reality higher than the results suggests but they are not really found in the water column but rather in the sediment or biota,
3) The toxicants really are more abundant but they are in different locations, or
4) The occurrence of toxicants is highly episodic and the current frequency of sampling is too low to detect all but the occasional peak.
5) The testing technology is not selective enough to assess the issues being addressed

Although option 1) may be true, a more conservative null hypothesis would be that the selected toxicants are present at significant, if not high, levels. This means that options 2 to 5 need to be addressed:

Sampling media
It is clear that an integrated assessment approach needs to be followed. To this end, it is recommended that trace organic content of sediment and biota be investigated as a matter of urgency. This investigation should not only establish the relevant methodology but also the optimal frequency of sampling and which environmental media is the most appropriate to estimate the immediate and long term status. In the longer terms a workable model relating the levels of toxicants in the various media needs to be investigated to aid interpretation. A concept note on the development of a solids monitoring programme in included in Appendix 4.

Choice of sampling sites
The choice of sampling sites will remain a subjective exercise for the foreseeable future. Ideally a national survey should have established the areas in which high toxicant concentrations or high toxicity is found. At present these sites are selected based on perceived potential. However, the sites need to be characterized carefully based on sound insight regarding their flow and concentration patterns. It is conceivable that different sites may yield different toxicants of concern and distinctive toxicity patterns. Consequently the next step would be to perform an intensive assessment at each proposed site so as to better understand the relationships among physical variables and the various interaction at the site.

Frequency of sampling
One of the issues that need attention in site characterization is high frequency sampling for short periods of time. Until the temporal response variability at a site is reasonably established, the sampling frequency cannot be determined. Based on hypothesised mechanisms of transport of toxicants to a given site (e.g. wash-off and/or dilution) a sampling strategy suitable to characterize this mechanism can be implemented for relatively short periods.

While these data are being collected, it is recommended that selected periods of high intensity sampling are used in order to characterize the variability in the resource. For example, after examining the estimated run-off characteristics at a point in a stream and making some assumptions on the origin of contaminants, it should be possible to select a frequency for high and low/base flow conditions that might suitably characterize the variability at that sampling point.

Trace elements and other organics
The focus in the original design of the NTMP was on toxicity and selected trace organic toxicants. There is at present no systematic study on the occurrence of trace elements and particularly heavy metals in our river systems. Internationally there are several institutions giving attention to the occurrence of mercury due to its toxicity and mobility in the environment. It is therefore proposed that trace elements be included in the list of variables for the NTMP. The proposed list is: V, Mn, Ni, Cu, Zn, As, Se, Sr, Cd, Sn, Sb, W, Hg, Pb, Bi and U(all dissolved and total) as well as Cr(VI). In order to interpret these data it is also necessary to measure the alkaline and alkaline earth metals as well as pH and major anion concentrations.

There are also a number of other trace organic compounds that would need to be evaluated for inclusion into the NTMP. Pesticides that are more labile than the POPs have been found like dimethoate in this study, but there are several more that are commonly used. These are not necessarily recalcitrant, but on a seasonal basis they may cause significant problems. At the time of writing there is a research project under way that looks for (particularly veterinary) pharmaceuticals in the water resource and the preliminary results (Dr James Meyer, researcher, University of Pretoria, personal communication) suggests that this is an area of concern.

Once sufficient confidence in the distribution characteristics of variables have been established the frequency of sampling can be decreased and the statistical distribution parameter trends can be tracked over time by using Bayesian techniques.

Until such time as the South African Water quality Guidelines have been updated, it would be fitting if the results are published either un-interpreted or interpreted using the relevant ANZECC guidelines (ANZECC, 1999) when required.

Research and development work on test methodologies and result assessment
Ideally, rapid and sensitive methods that yield continuous responses to contaminants should be considered. Focussed investigations that pronounce on the most suitable methods and their interpretation in terms of the national classification system are required as a matter of priority. In this process the focus should ideally be on methodology that is viable for in-house application (to the extent possible) and, as a corollary, there should be a strong focus on in-house capacity building. In-house capacity development would tend to reduce effects due personnel turn over, ensure some level of continuity and reduce expenditure in the deployment and maintenance of the NTMP. At the same time it is vitally important that scientific credibility be given the highest priority.

The development of integrative chemical samplers for use in the NTMP might also merit attention. An integrative sampler is a device designed in such a way that the water that flows through it is brought into contact with material that adsorbs the target spectrum of substances. By suitable calibration and with some knowledge of the flow an estimate can be made of the amount of material that passed the point in the stream at which it was installed. There is a substantial amount of development and possibly even research work that needs to be invested in this. Its advantage is that it addresses

to a certain extent the concern that toxicant “peaks” might be missed in the sampling regime.

The way ahead

While a certain measure of momentum has been gained during phase 3, this should be maintained to the extent possible. As a matter of urgency sites need to be identified that are suitable to represent the toxicity status of the national water resource using the guiding principles set out in DWAF (2005). The most suitable sites in the short term might be those in the Jukskei and Klip Rivers. On the longer term other suitable sites should be identified and systematically characterized.

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Appendix 1: Raw data

A summary of the raw data as existing on the WMS data base as at 15 June 2008. The data were separated into values ≤ detection limit (≤dl), values > detection limit (>dl) and missing data. The symbol "<" indicates "less than detection limit".

Station	MIDRAND (N=115)					JAGSPRUIT (N=26)				MARLBORO (N=125)				N14 (N=117)				KLEINSPRUIT (N=18)				KLIPRIVER (N=19)			
Variable	dl	≤dl	>dl	50	95	≤dl	>dl	50	95	≤dl	>dl	50	95	≤dl	>dl	50	95	≤dl	>dl	50	95	≤dl	>dl	50	95
AceN	0.001	56	6	<	0.009	4	0	<	<	54	11	<	0.015	54	7	<	0.0058	5	0	<	<	2	0	<	<
Ald	0.001	1	0	<	<	18	1	<	0.012	1	1	0.014	0.026	1	0	<	<	4	0	<	<	7	0	<	<
Aro	0.001	58	3	<	0.003	4	0	<	<	63	2	<	<	56	5	<	0.009	4	0	<	<	2	0	<	<
Atr	0.004	16	71	0.056	0.191	2	23	0.041	0.28	20	68	0.055	0.18	14	73	0.073	0.248	1	8	0.06	1.85	0	9	0.05	0.366
BaPyr	0.001	60	2	<	<	4	0	<	<	61	3	<	0.009	59	1	<	<	4	0	<	<	2	0	<	<
BuBePth	0.001	50	12	<	0.202	4	1	<	0.252	49	17	<	0.152	38	25	<	0.347	4	1	<	0.037	2	0	<	<
DBF	0.01	40	48	0.013	0.044	16	7	<	0.02	24	69	0.025	0.104	46	39	<	0.062	6	1	<	0.014	7	1	<	0.011
DDD	0.001	1	6	0.012	0.019	12	9	<	0.028	1	5	0.011	0.033	1	9	0.013	0.06	4	1	<	0.012	5	3	<	0.015
DDE	0.001	3	7	0.01	0.016	15	5	<	0.011	5	10	0.008	0.012	2	9	0.011	0.047	4	1	<	0.005	6	2	<	0.01
DDT	0.001	61	2	<	<	18	2	<	0.172	63	2	<	<	58	3	<	0.047	5	3	<	0.04	5	1	<	0.1
DPChMor	10	0	55	40	100	0	15	60	100	0	51	10	100	0	56	60	100	0	6	20	60	0	5	40	50
DPGro	10	0	12	28.7	47.8	0	3	21.1	24.3	0	10	29.4	67.6	0	6	24.3	35.9	0	2	10	10	0	2	10	10
DPMor	10	0	110	10	85	0	23	10	55.8	0	103	10	100	0	108	27.5	100	0	18	10	10	0	17	10	10
DPRep	871	41	0	<	<	11	0	<	<	42	0	<	<	32	0	<	<	5	0	<	<	4	0	<	<
Di3HB	0.001	2	0	<	<	14	2	<	0.023	1	0	<	<	1	0	<	<	3	0	<	<	4	0	<	<
Di4HB	0.001	1	0	<	<	15	1	<	0.021	1	0	<	<	1	0	<	<	3	0	<	<	4	0	<	<
DiBuPth	0.021	6	82	0.232	1.15	4	11	0.165	2	7	84	0.172	2.08	5	81	0.362	2.13	5	2	<	0.714	2	3	0.132	0.725
DiChlor	0.001	62	0	<	<	19	0	<	<	63	3	<	0.002	60	3	<	0.001	6	0	<	<	6	0	<	<
DiEtPth	0.023	17	72	0.054	0.247	6	7	0.048	0.315	19	70	0.061	0.423	14	76	0.079	0.24	5	2	<	0.086	2	2	<	0.097
DiHPth	0.001	53	8	<	0.02	4	0	<	<	52	12	<	0.029	50	11	<	0.044	5	0	<	<	2	0	<	<
DiMePth	0.004	40	40	<	0.118	7	6	<	0.053	38	46	0.008	0.121	30	47	0.011	0.119	4	1	<	0.134	4	1	<	0.01
DiMeth	0.001	56	6	<	0.038	18	1	<	0.087	59	5	<	0.035	58	3	<	0.0019	7	0	<	<	6	0	<	<
DiOctPth	0.001	53	8	<	0.451	4	0	<	<	58	6	<	0.158	55	6	<	0.229	4	0	<	<	2	0	<	<
Diel	0.001	1	0	<	<	16	3	<	0.058	1	0	<	<	1	1	0.3	0.598	4	1	<	0.008	7	0	<	<
EnSulA	0.001	2	0	<	<	18	1	<	0.005	1	2	0.011	0.015	0	2	0.008	0.012	4	1	<	0.089	6	0	<	<
EnSulB	0.001	1	3	0.006	0.088	15	4	<	0.008	1	2	0.002	0.024	2	6	0.015	0.13	5	1	<	0.003	7	0	<	<
EnSulSO4	0.001	1	1	0.005	0.008	19	0	<	<	1	1	0.025	0.049	1	0	<	<	5	0	<	<	7	0	<	<
End	0.001	1	0	<	<	19	0	<	<	1	0	<	<	1	0	<	<	4	1	<	0.003	7	0	<	<

Variable	dl	≤dl	>dl	50	95	≤dl	>dl	50	95	≤dl	>dl	50	95	≤dl	>dl	50	95	≤dl	>dl	50	95	≤dl	>dl	50	95
Fluo	0.004	27	58	0.011	0.05	5	9	0.008	0.017	21	67	0.0155	0.053	26	58	0.01	0.051	4	3	<	0.008	3	2	<	0.012
GupMor	10	0	79	10	21	0	20	10	80	0	78	10	10	0	79	10	40	0	15	10	25	0	14	10	10
HCB	0.001	9	0	<	<	14	2	<	0.041	8	1	<	0.013	8	1	<	0.002	7	0	<	<	6	0	<	<
Hep	0.001	1	0	<	<	19	0	<	<	0	2	0.858	1.7	1	0	<	<	5	0	<	<	6	1	<	0.008
HepO	0.001	1	0	<	<	18	1	<	0.002	1	0	<	<	1	0	<	<	3	1	<	0.02	6	1	<	0.002
MCPA	0.001	59	0	<	<	4	0	<	<	60	0	<	<	57	0	<	<	5	0	<	<	2	0	<	<
Mir	0.001	1	0	<	<	19	0	<	<	1	0	<	<	1	4	0.013	0.019	3	0	<	<	7	0	<	<
MonCrot	0.001	1	0	<	<	19	0	<	<	1	0	<	<	1	0	<	<	5	0	<	<	7	0	<	<
Napth	0.001	31	58	0.01	0.215	6	6	0.003	0.135	24	67	0.022	0.426	31	58	0.015	0.29	5	0	<	<	3	2	<	0.012
NonPhen	0.02	59	0	<	<	4	0	<	<	59	1	<	<	57	0	<	<	5	0	<	<	2	0	<	<
Phen	0.01	41	48	0.014	0.039	6	6	<	0.027	29	63	0.023	0.069	34	52	0.014	0.051	5	0	<	<	3	1	<	0.018
SelInhib	10	1	106	81.6	141	0	23	35.5	94	1	100	79.5	155	5	102	64	110	0	17	41.9	119	0	16	55.7	189
Sim	0.001	0	37	0.164	3.9	3	22	0.118	0.554	1	39	0.128	3.89	0	33	0.164	1.69	1	6	0.03	1.21	1	8	0.057	0.729
TerBut	0.001	2	14	0.059	0.202	6	15	0.055	1.49	1	17	0.074	0.154	1	14	0.072	0.308	0	7	0.06	1.15	0	9	0.04	0.193
Tet4HB	0.001	1	0	<	<	16	0	<	<	1	0	<	<	1	0	<	<	3	0	<	<	4	0	<	<
Tox	0.001	1	0	<	<	17	0	<	<	1	0	<	<	1	0	<	<	3	0	<	<	4	0	<	<
TriC4HB	0.001	1	0	<	<	16	0	<	<	1	0	<	<	1	0	<	<	3	0	<	<	4	0	<	<
Vibr	10	0	110	41	62.5	0	23	38.2	56.25	0	104	40.2	60	0	110	36.4	56.4	0	18	45.2	62.9	0	17	42.4	51.7
ZebMor	10	0	33	10	31.25	0	4	10	10	0	27	10	13.8	0	33	10	43.5	0	3	10	10	0	3	10	10
aBHC	0.015	9	79	0.274	1.63	21	2	<	0.031	65	11	<	0.13	31	57	0.039	0.338	7	1	<	0.024	6	2	<	0.045
aChlor	0.001	1	0	<	<	18	1	<	0.003	1	2	0.006	0.011	1	0	<	<	5	0	<	<	7	0	<	<
bBHC	0.004	12	70	0.301	1.1	16	4	<	0.116	53	12	<	0.365	32	43	0.04	0.236	6	1	<	0.018	5	2	<	0.041
dBHC	0.006	39	24	<	0.501	16	4	<	0.059	61	7	<	0.018	46	21	<	0.175	5	2	<	0.05	4	3	<	0.066
gBHC	0.006	34	42	0.01	0.066	12	9	<	0.054	48	31	<	0.092	24	63	0.039	0.233	3	5	0.018	0.031	4	5	0.012	0.056
gChlor	0.001	0	0	<	<	19	0	<	<	1	0	<	<	1	0	<	<	5	0	<	<	6	1	<	0.003
opD	0.001	0	0	<	<	4	0	<	<	60	0	<	<	58	0	<	<	4	0	<	<	2	0	<	<

A lognormal distribution with mean $\ln(\mu_l)$ and $\ln(\sigma)$ and an exponential distribution with mean μ_e was then fit to the data using maximum likelihood methods accommodating censored data.

Station	***MIDRAND (N=115)***					***MARLBORO (N=125)***					***N14 (N=117)***				
Statistic	***≤dl***	***>dl***	μ_l	σ	μ_e	***≤dl***	***>dl***	μ_l	σ	μ_e	***≤dl***	***>dl***	μ_l	σ	μ_e
AceN	56	6	0.01	2.15	0.02	54	11	0.01	2.13	0.02	54	7	0.02	4.25	0.11
Ald	1	0	NA	NA	NA	1	1	0.03	1.00	0.03	1	0	NA	NA	NA

Station	*MIDRAND (N=115)*					*MARLBORO (N=125)*					*N14 (N=117)*				
Statistic	*≤dl*	*>dl*	μ_l	σ	μ_e	*≤dl*	*>dl*	μ_l	σ	μ_e	*≤dl*	*>dl*	μ_l	σ	μ_e
Aro	58	3	0.09	6.54	0.52	63	2	0.01	1.98	0.05	56	5	0.04	5.07	0.27
Atr	16	71	0.06	1.96	0.08	20	68	0.07	2.01	0.08	14	73	0.08	2.09	0.11
BaPyr	60	2	0.06	1.55	0.09	61	3	0.06	2.41	0.10	59	1	0.04	1.00	0.10
BuBePth	50	12	0.06	3.19	0.12	49	17	0.06	2.47	0.09	38	25	0.08	3.43	0.16
DBF	40	48	0.03	2.11	0.07	24	69	0.04	1.96	0.05	46	39	0.02	1.67	0.03
DDD	1	6	0.01	1.51	0.01	1	5	0.01	1.69	0.02	1	9	0.02	2.01	0.02
DDE	3	7	0.01	1.89	0.01	5	10	0.01	1.38	0.01	2	9	0.01	1.62	0.02
DDT	61	2	0.22	1.25	0.26	63	2	0.05	3.77	0.128	58	3	0.49	3.26	0.87
DPChMor	0	55	29.3	2.72	45.1	0	51	24.4	2.70	38.8	0	56	33.8	2.96	54.1
DPGro	0	12	22.01	1.85	25.7	0	10	26.3	1.81	30.4	0	6	19.9	1.74	22.3
DPMor	0	110	13.00	1.95	18.4	0	103	13.6	2.11	20.7	0	108	29.3	2.87	47.7
DPRep	37	4	34.8	-860	>>	39	3	86.5	725	>>	30	2	71.1	>>	NA
Di3HB	2	0	NA	NA	NA	1	0	NA	NA	NA	1	0	NA	NA	NA
Di4HB	1	0	NA	NA	NA	1	0	NA	NA	NA	1	0	NA	NA	NA
DiBuPth	6	82	0.24	3.19	0.48	7	84	0.25	3.59	0.737	5	81	0.35	3.14	0.65
DiChlor	62	0	NA	NA	NA	63	3	0.01	2.01	0.04	60	3	0.06	5.17	0.18
DiEtPth	17	72	0.08	2.70	0.29	19	70	0.10	3.00	0.40	14	76	0.09	2.44	0.29
DiHPth	53	8	0.02	2.12	0.03	52	12	0.02	2.35	0.03	50	11	0.02	3.14	0.06
DiMePth	40	40	0.03	4.42	0.34	38	46	0.04	4.48	0.34	30	47	0.03	3.76	0.25
DiMeth	56	6	0.04	1.75	0.06	59	5	0.10	3.95	0.33	58	3	0.01	2.52	0.04
DiOctPth	53	8	0.28	3.00	0.43	58	6	0.19	2.59	0.31	55	6	0.12	6.11	0.37
Diel	1	0	NA	NA	NA	1	0	NA	NA	NA	1	1	0.60	1.00	0.60
EnSulA	2	0	NA	NA	NA	1	2	0.01	1.17	0.01	0	2	0.01	2.18	0.01
EnSulB	1	3	0.01	3.97	0.03	1	2	0.01	3.30	0.01	2	6	0.02	3.11	0.04
EnSulSO4	1	1	0.01	1.000	0.01	1	1	0.05	1.00	0.05	1	0	NA	NA	NA
End	1	0	NA	NA	NA	1	0	NA	NA	NA	1	0	NA	NA	NA
Fluo	27	58	0.02	1.93	0.02	21	67	0.02	2.47	0.06	26	58	0.02	2.21	0.03
GupMor	0	79	10.7	1.37	11.6	0	78	10.5	1.33	11.3	0	79	11.0	1.44	12.2
HCB	9	0	NA	NA	NA	8	1	0.01	1.00	0.02	8	1	0.002	1.00	0.01
Hep	1	0	NA	NA	NA	0	2	0.16	28.4	0.86	1	0	NA	NA	NA
HepO	1	0	NA	NA	NA	1	0	NA	NA	NA	1	0	NA	NA	NA
MCPA	59	0	NA	NA	NA	60	0	NA	NA	NA	57	0	NA	NA	NA
Mir	1	0	NA	NA	NA	1	0	NA	NA	NA	1	4	0.01	1.41	0.02
MonCrot	1	0	NA	NA	NA	1	0	NA	NA	NA	1	0	NA	NA	NA
Napth	31	58	0.03	3.96	0.07	24	67	0.05	4.97	0.15	31	58	0.05	3.88	0.10
NonPhen	59	0	NA	NA	NA	59	1	0.26	1.00	0.85	57	0	NA	NA	NA
Phen	41	48	0.02	1.60	0.03	29	63	0.03	1.67	0.04	34	52	0.02	1.60	0.03
SelInhib	1	106	72.0	1.75	81.7	1	100	77.4	1.63	86.0	5	102	59.8	1.73	67.7
Sim	0	37	0.14	5.58	0.54	1	39	0.21	4.17	0.70	0	33	0.15	4.02	0.35
TerBut	2	14	0.07	1.97	0.09	1	17	0.06	1.92	0.08	1	14	0.09	1.84	0.11
Tet4HB	1	0	NA	NA	NA	1	0	NA	NA	NA	1	0	NA	NA	NA
Tox	1	0	NA	NA	NA	1	0	NA	NA	NA	1	0	NA	NA	NA
TriC4HB	1	0	NA	NA	NA	1	0	NA	NA	NA	1	0	NA	NA	NA

Station	*MIDRAND (N=115)*					*MARLBORO (N=125)*					*N14 (N=117)*				
Statistic	***≤dl***	***>dl***	μ_l	σ	μ_e	***≤dl***	***>dl***	μ_l	σ	μ_e	***≤dl***	***>dl***	μ_l	σ	μ_e
Vibr	0	110	36.1	1.61	39.6	0	104	34.7	1.61	38.1	0	110	28.4	1.86	33.2
ZebMor	0	33	10.8	1.38	11.7	0	27	10.5	1.27	10.9	0	33	12.1	1.62	14.2
aBHC	9	79	0.29	3.50	0.82	65	11	0.09	2.74	0.21	31	57	0.08	2.65	0.19
aChlor	1	0	NA	NA	NA	1	2	0.01	1.35	0.01	1	0	NA	NA	NA
bBHC	12	70	0.34	2.95	0.73	53	12	0.13	6.12	1.33	32	43	0.09	2.17	0.13
dBHC	39	24	0.10	3.06	0.18	61	7	0.02	1.83	0.05	46	21	0.05	2.89	0.10
gBHC	34	42	0.03	2.26	0.05	48	31	0.03	2.18	0.05	24	63	0.05	2.08	0.07
gChlor	1	0	NA	NA	NA	1	0	NA	NA	NA	1	0	NA	NA	NA
opD	59	0	NA	NA	NA	60	0	NA	NA	NA	58	0	NA	NA	NA

Station	*JAGSPRUIT (N=26)*					*KLEINSPRUIT (N=18)*					*KLIPRIVER (N=19)*				
Statistic	***≤dl***	***>dl***	μ_l	σ	μ_e	***≤dl***	***>dl***	μ_l	σ	μ_e	***≤dl***	***>dl***	μ_l	σ	μ_e
AceN	4	0	NA	NA	NA	5	0	NA	NA	NA	2	0	NA	NA	NA
Ald	18	1	0.02	1.00	0.04	4	0	NA	NA	NA	7	0	NA	NA	NA
Aro	4	0	NA	NA	NA	4	0	NA	NA	NA	2	0	NA	NA	NA
Atr	2	23	0.05	2.44	0.07	1	8	0.13	5.23	0.42	0	9	0.06	3.28	0.10
BaPyr	4	0	NA	NA	NA	4	0	NA	NA	NA	2	0	NA	NA	NA
BuBePth	4	1	0.25	1.00	0.26	4	1	0.04	1.00	0.04	2	0	NA	NA	NA
DBF	16	7	0.01	1.29	0.03	6	1	0.01	1.00	0.04	7	1	0.01	1.00	0.05
DDD	12	9	0.01	1.90	0.02	4	1	0.01	1.00	0.02	5	3	0.01	1.14	0.01
DDE	15	5	0.01	1.34	0.01	4	1	0.01	1.00	0.01	6	2	0.01	1.58	0.01
DDT	18	2	0.11	2.75	0.18	5	3	0.02	1.67	0.03	5	1	0.10	1.00	0.11
DPChMor	0	15	32.2	2.80	48.7	0	6	21.2	2.35	28.3	0	5	25.1	2.33	32.0
DPGro	0	3	17.2	1.61	18.5	0	2	10.0	1.00	10.0	0	2	10.0	1.00	10.0
DPMor	0	23	11.7	1.68	14.6	0	18	10.0	1.00	10.0	0	17	10.0	1.00	10.0
DPRep	9	2	33.0	-500	>>	2	3	64.0	87.8	73.7	2	2	66.3	-75.0	71.5
Di3HB	14	2	0.02	1.12	0.04	3	0	NA	NA	NA	4	0	NA	NA	NA
Di4HB	15	1	0.03	1.000	0.04	3	0	NA	NA	NA	4	0	NA	NA	NA
DiBuPth	4	11	0.34	3.04	0.60	5	2	0.46	1.55	0.53	2	3	0.30	2.01	0.39
DiChlor	19	0	NA	NA	NA	6	0	NA	NA	NA	6	0	NA	NA	NA
DiEtPth	6	7	0.12	1.80	0.16	5	2	0.06	1.37	0.10	2	2	0.06	1.76	0.08
DiHPth	4	0	NA	NA	NA	5	0	NA	NA	NA	2	0	NA	NA	NA
DiMePth	7	6	0.012	2.08	0.02	4	1	0.13	1.00	0.14	4	1	0.01	1.00	0.02
DiMeth	18	1	0.16	1.00	0.12	7	0	NA	NA	NA	6	0	NA	NA	NA
DiOctPth	4	0	NA	NA	NA	4	0	NA	NA	NA	2	0	NA	NA	NA
Diel	16	3	0.02	3.32	0.04	4	1	0.01	1.00	0.01	7	0	NA	NA	NA
EnSulA	18	1	0.01	1.000	0.03	4	1	0.10	1.00	0.09	6	0	NA	NA	NA
EnSulB	15	4	0.01	1.35	0.01	5	1	0.003	1.00	0.01	7	0	NA	NA	NA
EnSulSO4	19	0	NA	NA	NA	5	0	NA	NA	NA	7	0	NA	NA	NA
End	19	0	NA	NA	NA	4	1	0.003	1.00	0.01	7	0	NA	NA	NA
Fluo	5	9	0.01	1.42	0.01	4	3	0.01	1.05	0.01	3	2	0.01	1.41	0.01
GupMor	0	20	13.4	2.07	19.5	0	15	10.8	1.33	11.3	0	14	10.0	1.00	10.0

Station	*JAGSPRUIT (N=26)*					*KLEINSPRUIT (N=18)*					*KLIPRIVER (N=19)*				
Statistic	*≤dl*	*>dl*	μ_l	σ	μ_e	*≤dl*	*>dl*	μ_l	σ	μ_e	*≤dl*	*>dl*	μ_l	σ	μ_e
HCB	14	2	0.02	3.84	0.04	7	0	NA	NA	NA	6	0	NA	NA	NA
Hep	19	0	NA	NA	NA	5	0	NA	NA	NA	6	1	0.01	1.00	0.01
HepO	18	1	0.002	1.000	0.02	3	1	0.02	1.00	0.02	6	1	0.002	1.000	0.01
MCPA	4	0	NA	NA	NA	5	0	NA	NA	NA	2	0	NA	NA	NA
Mir	19	0	NA	NA	NA	3	0	NA	NA	NA	7	0	NA	NA	NA
MonCrot	19	0	NA	NA	NA	5	0	NA	NA	NA	7	0	NA	NA	NA
Napth	6	6	0.02	3.21	0.04	5	0	NA	NA	NA	3	2	0.01	1.10	0.01
NonPhen	4	0	NA	NA	NA	5	0	NA	NA	NA	2	0	NA	NA	NA
Phen	6	6	0.02	1.28	0.02	5	0	NA	NA	NA	3	1	0.02	1.00	0.03
SelInhib	0	23	33.3	2.13	42.0	0	17	36.8	1.95	44.8	0	16	39.3	2.82	60.3
Sim	3	22	0.09	4.37	0.18	1	6	0.11	7.20	0.42	1	8	0.09	4.34	0.20
TerBut	6	15	0.13	4.43	0.37	0	7	0.13	4.89	0.33	0	9	0.04	3.54	0.08
Tet4HB	16	0	NA	NA	NA	3	0	NA	NA	NA	4	0	NA	NA	NA
Tox	17	0	NA	NA	NA	3	0	NA	NA	NA	4	0	NA	NA	NA
TriC4HB	16	0	NA	NA	NA	3	0	NA	NA	NA	4	0	NA	NA	NA
Vibr	0	23	35.9	1.58	38.8	0	18	39.7	1.57	42.8	0	17	37.3	1.32	38.6
ZebMor	0	4	10.00	1.00	10.00	0	3	10.0	1.00	10.0	0	3	10.0	1.00	10.0
aBHC	21	2	0.03	1.12	0.12	7	1	0.02	1.00	0.08	6	2	0.04	1.04	0.07
aChlor	18	1	0.004	1.000	0.02	5	0	NA	NA	NA	7	0	NA	NA	NA
bBHC	16	4	0.035	3.215	0.070	6	1	0.02	1.00	0.03	5	2	0.02	2.46	0.03
dBHC	16	4	0.030	1.992	0.050	5	2	0.04	1.20	0.05	4	3	0.06	1.14	0.06
gBHC	12	9	0.019	2.160	0.029	3	5	0.02	1.37	0.03	4	5	0.03	1.64	0.03
gChlor	19	0	NA	NA	NA	5	0	NA	NA	NA	6	1	0.003	1.00	0.01
opD	4	0	NA	NA	NA	4	0	NA	NA	NA	2	0	NA	NA	NA

aChlor	Chlordane *cis* (alpha)	Sim	Simazine	BuBePth	Butylbenzylphtalate
gChlor	Chlordane *trans* (gamma)	MonCrot	Monocrotophos	DiOctPth	Di-*n*-octylphthalate
Di4HB	2,5-dichloro-4-hydroxybiphenyl	Atr	Atrazine	HCB	Hexachlorobenzene
Tet4HB	2,3,4,5-tetrachloro-4-hydroxybiphenyl	opD	2,4-dichlorophenoxy acetic acid	Napth	Naphtalene
TriC4HB	2,4,6-trichloro-4-hydroxybiphenyl	TerBut	Terbuthylazine	DPGro	Daphnia pulex % growth, stimulation
AceN	Acenaphthylene	EnSulSO4	Endosulfan sulfate	DPMor	Daphnia pulex % mortality
Di3HB	2,5-dichloro-3-hydroxybiphenyl	EnSulA	Endosulfan-a	DPRep	Daphnia pulex % reproduction
Ald	Aldrin	EnSulB	Endosulfan-b	DPChMor	Daphnia pulex % reproduction, mortality
Diel	Dieldrin	MCPA	MCPA (metaxon)	ZebMor	Danio rerio embryos and larvae % mortality
End	Endrin	BaPyr	Benzo(*a*)pyrene	GupMor	Poecilia reticulata , % mortality
DDD	DDD-4,4	DiMePth	Dimethyl phtalate	SelInhib	Selenastrum capricornutum, % stimulation
DDE	DDE-4,4	DiEtPth	Diethylphthalate	Vibr	Vibrio fischeri, % stimulation

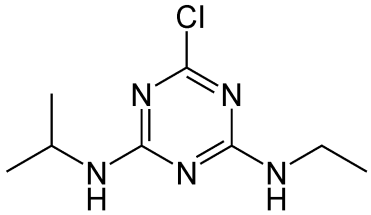
DiHPth	Di-n-hexyl phthalate	DBF	Dibenzofuran	NonPhen	Nonyl phenol
DiChlor	Dichlorvos	Phen	Phenanthrene	DiBuPth	Di-*n*-butylphtalate
DiMeth	Dimethoate	Fluo	Fluoranthene		

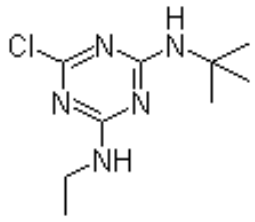
Percentile analysis on pooled data

Variable	***dl***	***Percentiles***																		
		0.05	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**	**0.35**	**0.4**	**0.45**	**0.50**	**0.55**	**0.6**	**0.65**	**0.7**	**0.75**	**0.8**	**0.85**	**0.9**	**0.95**
2,4D	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Acenaphthylene	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.003	0.011
Aldrin	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.008
Arochlor	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Atrazine	0.004	<dl	<dl	<dl	0.014	0.022	0.030	0.041	0.049	0.054	0.057	0.062	0.070	0.078	0.084	0.094	0.107	0.138	0.175	0.229
Benzo(a)pyrene	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
BHC alpha	0.015	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.024	0.035	0.049	0.068	0.096	0.164	0.237	0.355	0.489	0.816
BHC beta	0.004	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.011	0.040	0.060	0.085	0.136	0.168	0.253	0.415	0.587	0.777
BHC delta	0.006	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.010	0.022	0.040	0.065	0.139
BHC gamma (Lindane)	0.006	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.007	0.012	0.020	0.023	0.026	0.030	0.036	0.043	0.052	0.062	0.104
Bipheny, l2,4,6-trichloro-4-hydroxy	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Biphenyl, 2,3,4,5-tetrachloro-4-hydroxy	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Biphenyl, 2,5-dichloro-3-hydroxy	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.001	0.001	0.014
Biphenyl, 2,5-dichloro-4-hydroxy	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Chlordane cis (alpha)	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.005
Chlordane trans (gamma)	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
D. pulex % growth, stimulation	10	<dl	<dl	<dl	<dl	<dl	<dl	<dl	21.1	22.0	24.3	26.0	27.6	29.4	30.7	33.0	35.3	35.8	37.2	42.4
D. pulex % mortality	10	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	40.000	75.000	95.000	100.000
D. pulex % reproduction	10	-550	-369	-300	-216	-168	-148	-115	-101	-93.1	-88.0	-76.6	-67.0	-58.9	-41.4	-33.0	-10.0	8.0	28.8	59.3

D. pulex % reproduction, mortality	10	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	35.0	50.0	60.0	60.0	70.0	82.5	100	100	100	100
D. rerio embryos and larvae % mortality	10	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	34.000
DDD-4,4	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.005	0.007	0.007	0.011	0.011	0.012	0.013	0.015	0.018	0.021	0.029
DDD-4,4	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.004	0.008	0.009	0.010	0.010	0.011	0.011	0.012	0.014
DDT, 4-,4'-,	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.011
Dibenzofuran	0.01	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.012	0.015	0.018	0.022	0.024	0.026	0.029	0.036	0.047	0.064
Dichlorvos	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Dieldrin	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.006	0.036
Dimethoate	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.025
Endosulfan sulfate	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.003
Endosulfan-a	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.003	0.009	0.013
Endosulfan-b	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.003	0.004	0.006	0.009	0.020	0.034
Endrin	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Fluoranthene	0.004	<dl	<dl	<dl	<dl	<dl	<dl	0.005	0.006	0.009	0.010	0.012	0.014	0.017	0.018	0.020	0.022	0.027	0.033	0.049
Heptachlor	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.010
Heptachlor epoxide	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.002
Hexachlorobenzene	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.002
Mcpa (metaxon)	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Mirex	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.005	0.014
Monocrotophos	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Naphtalene	0.001	<dl	<dl	<dl	<dl	<dl	<dl	0.002	0.004	0.009	0.013	0.016	0.021	0.029	0.042	0.065	0.094	0.140	0.215	0.352
Nonyl phenol	0.02	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
P. reticulata , % mortality	10	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	26.000
Phenanthrene	0.01	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.013	0.014	0.016	0.018	0.021	0.024	0.026	0.030	0.036	0.044	0.054
Phtalate, butylbenzyl	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.013	0.038	0.057	0.111	0.251
Phtalate, dimethyl	0.004	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.007	0.009	0.010	0.012	0.015	0.021	0.032	0.060	0.072	0.119
Phtalate,di-n-butyl	0.021	<dl	0.024	0.043	0.056	0.084	0.105	0.137	0.162	0.202	0.244	0.289	0.361	0.430	0.487	0.585	0.716	0.839	1.088	1.670
Phthalate, diethyl	0.023	<dl	<dl	<dl	<dl	0.026	0.032	0.039	0.047	0.054	0.061	0.069	0.077	0.084	0.096	0.106	0.115	0.134	0.186	0.279
Phthalate, di-n-hexyl	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.003	0.012	0.024
Phthalate, di-n-	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	0.003	0.284

octyl																				
S. capricornutum, % stimulation	10	<dl	25.000	33.940	41.744	45.910	51.195	54.192	60.434	65.633	69.810	74.895	79.505	84.045	91.394	95.765	100.590	107.955	122.605	139.745
Simazine	0.001	0.003	0.010	0.025	0.036	0.057	0.068	0.080	0.102	0.117	0.135	0.175	0.231	0.263	0.285	0.348	0.425	0.571	0.813	1.410
Terbuthylazine	0.001	<dl	<dl	0.018	0.020	0.035	0.038	0.041	0.050	0.056	0.065	0.074	0.085	0.091	0.106	0.118	0.158	0.190	0.285	0.685
Toxaphene	0.001	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
V. fischeri, % stimulation	10	<dl	<dl	22.924	25.398	27.308	30.633	32.916	34.936	37.073	39.120	40.991	42.052	43.792	45.444	47.488	49.224	51.000	55.400	59.465


Atrazine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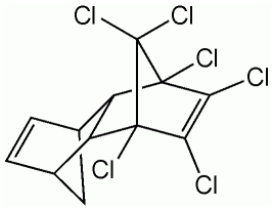
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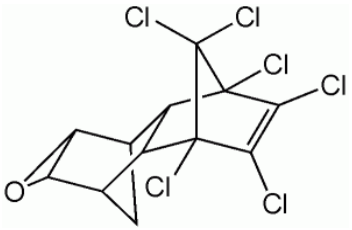
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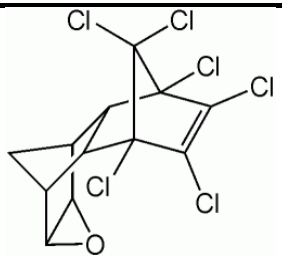
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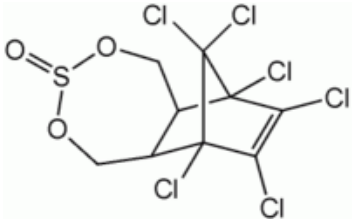
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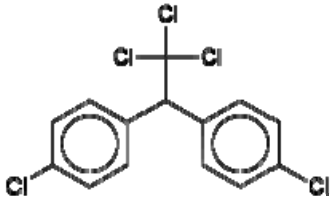
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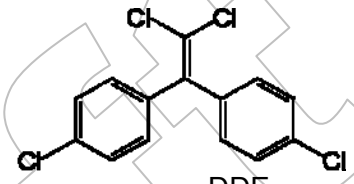
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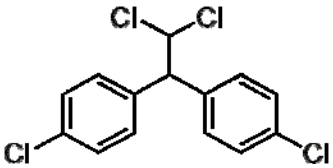
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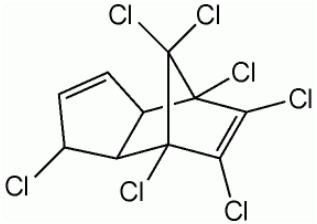
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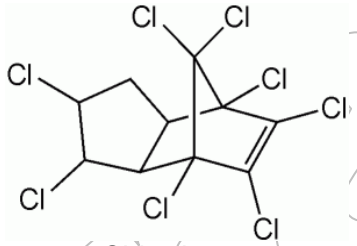
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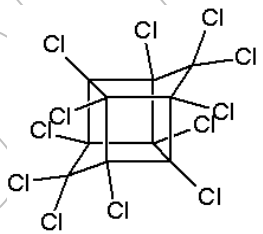
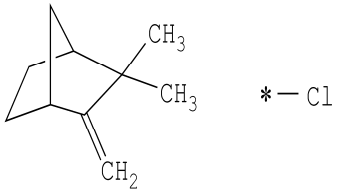
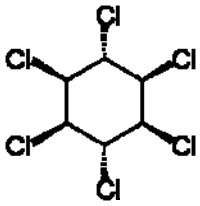
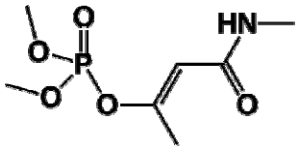

Mirex


Toxaphene



γBHC



Monocrotophos

Appendix 2: Costing

The projected costs using the model proposed in DWAF 2005. This provides the estimated cost if the project had been run as scheduled.

				Annual Running costs	**%**
	Monitoring points				
NP	**6**	**Number of monitoring points**		**R 798,292**	**25 Transport**
NR	**52**	**No. of visits per year (to all points)**		**R 2,449,200**	**75 Analytical**
	312	Number of samples per year		**R 3,247,492**	**TOTAL**
	5	**No. days over which sampling is spread**			
	Transport costs			**R 10,409**	**R/sample**
	R 9.86	**Petrol costs (R/litre)**			
	8	**Fuel consumption (litres/100 km) for sampling vehicle**		**Lab Capital Setup Costs**	
	R 2.37	**Sampling vehicle fuel & maintenance costs (Rands) / km**		**R 0**	**Toxicity**
	R 0.00	**Toxicity**	**Courier costs (Rands) for samples / sample / km**	**R 0**	**Organics**
	R 0.00	**Organics**	**Courier costs (Rands) for samples / sample / km**		
	R 3.16	Sample transport costs (Rands) / km (monitoring point to nearest town)		**No. of existing labs**	
	1	**Toxicity**	**Number of laboratories (max 5)**	**1**	**Toxicity**
	1	**Organics**	**Number of laboratories (max 5)**	**1**	**Organics**
	1.2	Toxicity	Samples / lab / day	**No. new labs needed**	
	1.2	Organics	Samples / lab / day	**0**	**Toxicity**
	312	Toxicity	Samples / lab / year	**0**	**Organics**
	312	Organics	Samples / lab / year	**Max. samples / lab / visit (to all points)**	
	6.0	Toxicity	Samples / lab / week	**10**	**Toxicity**
	Transport distances			**10**	**Organics**
d(town)	**810**	**Average distance (km) from monitoring point to nearest town**		**Min. samples / lab / yr (for financial vaibility)**	
d(tox)	**0**	**Toxicity**	**Average dist. (km) from town to nearest lab**	**0**	**Toxicity**
d(org)	**0**	**Organics**	**Average dist. (km) from town to nearest lab**	**0**	**Organics**
	Transport costs / year				
	R 798,292	Annual cost transport from mon. point to nearest town			
	R 0	Toxicity (Annual cost from town to nearest lab)			
	R 0	Organics (Annual cost from town to nearest lab)			

	Transport costs / year	
	R 798,292	R 0.79 **0.12**
	In	
	1	**Toxicity**
	1	**Fish**
	1	**Daphnia**
	1	**Algae**
	1	**Yeast**
	1	**Organics**
	1	**Non-POPs**
	Analytical costs	
	R 5,350	**Toxicity (Analytical costs / sample)**
	R 2,500	**Organics (Analytical costs / sample)**
	R 7,850	Total analytical costs / sample
	Analytical costs / year (calc'd)	
		R 2,449,200

Appendix 3: Comment on the Toxicity Testing Needs for the NTMP

Abstracts from: "Review Of Toxicity Testing In The National Toxicity Monitoring Programme (NTMP): After The Pilot Phase."by Melusi Thwala, Directorate: RQS (April 2008)

This report observed that:

- The occurrence of chemicals identified during the design phase at very low levels in the water column during the pilot phase, argues for the review of the target sampling medium in the NTMP. It is proposed that sediments be also included as the sampling media but before this is implemented an intensified trial sediment programme is required to determine applicability; sampling and analytical institutions around the country will have to be identified and other logistic issues addressed.
- Trace metals which were not analyzed in the trial phase should be considered as they are highly relevant for sediment chemistry and toxicity.
- Although biota tissue chemical analysis is highly suitable for the NTMP [objectives] it still falls short on the ease of monitoring factors. Regardless of such, there needs to be parallel investigations on how biota tissue analysis can be utilized in the programme since tissue analysis coupled with other media analysis would provide a significant data input in an evidence based [status assessment]. As an example tissue analysis need only be performed at low frequency to provide a status of tissue contamination by specific chemicals.
- Since it is difficult to find a suitable species distributed through out the country, different species can be selected belonging to the same trophic level or and with the same feeding strategy. The planning for these parallel investigations will require participation by other internal and external specialists (ecologists, analytical chemists, toxicologists).
- It is also suggested that water analysis be continued and then, at a later stage when data to compare sediment to water analysis are available, a decision be taken to select any or all tested media driven by information cost considerations.

In consideration of available bio-assessment technologies it observed:

Semi-field Experimental Systems [so called 'cosms' methodology]

- As per NTMP design requirements, 'cosms' can not as yet be considered as standard NTMP methodology because of lack of reproducibility, difficulty in interpretation, high running costs including and local lack skills in dealing with such exposures and difficulty in collecting and maintaining sampled biota.
- The NTMP aims at protecting ecosystem integrity by detecting and reporting early effects so that mitigation steps can possibly be taken. 'Cosms', being mainly based on community effects, might respond too slowly to support for mitigation steps and hence they might not necessary serve the purpose of ecosystem integrity protection. Effects observable at lower levels of organization are more suitable for the NTMP purposes; for risk assessment and motivation of early management actions.
- Semifield studies can still be used in parallel NTMP support investigations; if for example a significant toxicity effects are observed at the individual level, such experiments can be employed to provide more cause-effect evidence at community level which can be very useful in ecological risk assessment.

***In Situ* Exposures**

The running costs (developing, monitoring and analysis) of field exposures for a national programme are likely to be very high and such coupled with loss, vandalism and low quality assurance (lack of standard procedures) related to field studies justifies the continued exclusion of *in situ* experiments in the NTMP. Since there is generally good quality information surrounding field experiments, such experiments can still be utilized in parallel NTMP support investigations.

Biomarkers

- **MTs (metallothineins)**: There is currently a wide range of methods to measure tissue levels of MTs but mostly are based on protein metal saturation and reverse transcription polymerase chain reaction (RT-PCR) methods. The RT-PCR reportedly provides more specific information than metal saturation (quick and simple) but relatively more complex and is time consuming. MT assessment is generally not a highly complex procedure but is time consuming. Although MTs assessment is highly relevant when coupling metal analysis especially in the sediments, it is however not suitable for the NTMP due to its high turn around time when considering the programme's multi bioassay approach and a sampling frequency requiring relatively quick assays.
- **Esterases activity;** Acetylcholine esterase (AChE) activity has been shown to be sensitive to carbamates and organophosphate pesticides. This assay is one of the most widely used to detect exposure to organophosphates. Although there is no standardized protocol the Ellman procedure is the most widely accepted and applied procedure. This assay (AChE) is highly recommended for routine monitoring in the NTMP especially within a multi biomarker type of approach which includes general health biomarkers.
- **Mixed function oxygenases (MFOs) activity:** The activity of the MFOs is affected by a wide range of lipophillic xenobiotic substances including dioxins, PCBs and PAHs which the NTMP focuses on. EROD being a simple, widely and successfully applied method is highly recommended for inclusion in the NTMP to couple AChE. These biomarkers (EROD and AChE) should be accompanied by a general cellular health status e.g. cellular energy allocation.
- **Lysosomal membrane stability (LMS):** Serious QA/QC issues surrounding LMS especially for a large scale programme such as the NTMP makes it unsuitable for routine monitoring.
- **Heat shock protein (Hsp):** As a general stress biomarker, reproducibility of results has been shown as one of the most limiting factors on quantitative application. Because of its complexity and high turn over time its suitability for the NTMP is low as QA/QC is likely to be compromised.
- **Genotoxicity biomarkers:** Although gene expression is a very sensitive indicator of toxicity effects at molecular level, it is extremely difficult to interpret or relate the results to higher levels of organization. Genotoxicity responses are useful at higher tiers when responses at higher levels did not indicate significant effects. There are also high running costs associated with such tests in terms of specialized equipment and skills, such can not be sustainable on a scale of the NTMP. Therefore any gene reporter assays are currently not suitable for the programme.
- **Cellular Energy Allocation(CEA):** Is a good general stress biomarker especially when coupled with another specific biomarker. CEA be used in the NTMP as a general health status biomarker which will support and improve the quality (ecological relevance) provided by other sub lethal assays. With the use of multivariate statistics there is a possibility to identify which responses (assays) correlate highly to energy expenditure; such information is helpful in identifying priority problem toxicants.

- **Glutathione *S* Transferase (GST):** GST activity analysis was shown to be very useful in determining exposure and induction of toxicity by organic xenobiotics. The GST activity detects exposure to toxicants but may also indicate the organism's physiological response to minimize toxicity. The application of such an assay of exposure in the NTMP is recommended.
- **Endocrine disruptive compounds (EDCs):** Currently the VTG biomarker is widely used because it is simpler and more cost effective relative than gene dependant assays. A WRC study recommended VTG assessment as one of the suitable biomarkers for screening EDCs exposure. In South Africa there are laboratories capacitated to perform VTG assessment. A number of current research initiatives are aimed at cost effective methods to screen EDCs exposure and can be consulted on the suitable protocol for VTG assessment. There is a wide variety of genomic assays to screen exposure to EDCs but these are not recommended for the NTMP as mentioned for other genetic biomarkers above.

Appendix 4: Concept note on the development of a sediment monitoring programme

Abstract form: *Concept Note for the Design of a Sediment Monitoring Programme* by Silke Bollmohr (RQS) August 2007.

"Sediment is an essential, integral and dynamic part of our river basins. Where human activities interfere with sediment quantity and quality, sediment management becomes necessary. Especially in African countries erosion and therefore sedimentation is an important economical barrier e.g. in terms of loss of valuable soil within agriculture or decrease of capacity in dams. But sediments also accumulate contaminants and serve as sources of pollution to the ecosystems they are connected with.
"The management of water resources in South Africa, guided by the National Water Act (1998), places emphasis on the protection of the water resource as a whole so as to ensure waters remain fit for use on a sustainable basis. The water resource as a whole includes water quality and quantity, in-stream and riparian habitat, and in-stream and riparian biota. Water quality includes natural parameters as ph, Temperature and turbidity but also contaminants either dissolved in the water column or associated to particles. Therefore suspended solids are a crucial part of the water quality and needs to be addressed with a monitoring programme. The effects of sediments on receiving waters are complex and multi-dimensional, and further compounded by the fact that sediment flux is a natural and vital process for aquatic systems. Aquatic organisms in a natural environment exposed to sediment particles being impacted by either the chemical associated to the particles (sediment quality), by the particles as physical stressor (sediment quantity) or both, whereas the interaction of suspended solids and toxicants is not completely solved.

"In managing river water quality, not only point sources have great influence on water and sediment quality, but also diffuse sources of contamination from groundwater, runoff and especially from contaminated sediment (Heise and Ahlf, 2002). Contaminated sediments are important for the water quality of rivers because they prolong the residence time of pollutants in river basins by accumulating organic and inorganic contaminants, by retarding their transport in the river basin due to often decreased degradation of organic substrates under anoxic conditions. Many toxic and bioaccumulative chemicals (such as metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorophenols, organochlorine pesticides and polybrominated diphenyl ethers), are only found in trace amounts in water but can accumulate to elevated levels in sediments. A study performed by Resource Quality Services on levels of PCB's and Polychlorinated debenzodioxins and dibenzofurans (PCDD/PCDF) in the sediment and water at three different sites within Gauteng region found concentrations of PCB's and PCDD/PCDF's below detection limit in the water whereas the concentration in the sediment ranged from 0.19 – 1.19 µg/kg and 0.3 to 426 ng/kg. In addition to providing sinks for many chemicals, sediments can also serve as potential sources of pollutants to the water column when conditions change in the receiving water systems especially in dams, (e.g. during periods of anoxia, or after severe storms). The continuous exchange between sediment and water during settlement and resuspension phases of contaminated particles during transport has the potential to impact previously not or less contaminated areas. (Foerstner et al., 2004). Therefore the risk of bound contaminants being spread within the river basin, e.g. during situations of high water discharges growing with increasing amount of sediment trapped in a river basin.

"Contaminated sediment represents an important environmental concern for several reasons:

1. Contaminated sediments have been demonstrated to be toxic to sediment dwelling organisms and benthic fish, which could result in decreased survival (acute toxicity) or reduced growth, and impaired reproduction (chronic toxicity) in benthic fish and invertebrates.
2. Certain sediment-associated contaminants (bio accumulative substances) are bio accumulated by benthic organisms and are passed on to other organisms higher up in the food chain (called bio magnification).
3. Contaminated sediments can affect human health due to direct exposure when wading or swimming or drinking turbid water and indirect through consumption of contaminated fish and shellfish.

“Poor land-use activities, high irregular rainfall patters and certain soil types can result in high levels of erosion and sedimentation, resulting in high turbidity (sediment quantity). High levels of turbidity can either have a (1) direct or (2) indirect effect on biota: (1) Examples of direct effects on biota include suppression of photosynthesis by shading primary producers (Waters, 1995) increased drifting of, and consequent predation on, benthic invertebrates; and shift to turbidity-tolerant fish communities. Either the behaviour of fish can be affected by high levels of turbidity, such as inability to see prey or feed normally or the physiology can be affected, such as gill clogging. Indirect effects on biota will occur as the biotic assemblages that rely upon aquatic habitat for reproduction, feeding and cover are adversely affected by habitat loss or degradation of this habitat. Changes in the supply of sediment causes drastic changes in aquatic, wetland and riparian vegetation, which can be induced by both decreases and increases in TSS from natural levels. High levels of sedimentation in rivers and dams lead also to physical disruption of the hydraulic characteristics of the channel. This can lead to increased flooding because of reduction in capacity of the river channel or dam. Total suspended solids are highly variable temporal and spatial. The orange River e.g. shows a range of TSS from 1-275266 mg/l, whereas within a river system one would expect the highest amount of TSS in estuaries.

“However suspended sediments may also effect other users than the aquatic ecosystem. Since many suspended solids can be a carrier for contaminants the use of water with suspended solids for irrigation or cattle feeding purposes can have detrimental effects on crop growth and cattle health. Bio magnification especially is an issue which needs to be addressed when looking at transfer of particle associated contaminants from the drinking water for cattle, via the cattle to the human via meat or milk production. Also industry as a user can be impacted by high suspended solids in the water by various mechanism, which needs to be discussed.

“How would the monitoring programme link to current initiatives?
“National Toxicity Monitoring Programme (NTMP)
The NTMP, now at the end of the pilot phase, aims to assess the toxicity status of South African surface waters. The variables proposed include both chemicals in the water and a range of direct toxicity assessments of the water column. After a critical review of the results out of the pilot phase it was concluded that many of the selected variables will be expected to be associated to sediment particles due to no correlation between concentrations of pesticides and POPS and the toxicity of the water. Therefore it is recommended to include sediment as a media and sediment toxicity test to assess the toxicity status of South African surface waters.”