CHAPTER 6:
CHOICE OF MONITORING VARIABLES

6.1 INTRODUCTION

Having established in previous chapters the framework and some generic criteria for decision-making in respect of monitoring variables, this chapter summarises the actual decisions taken and their justification.

The choices relating to toxicity were made in a workshop attended mainly by local biotoxicologists. All agreed that the overall framework was satisfactory and general consensus was reached in respect of the specific design recommendations.

6.2 TOXICITY

6.2.1 Specific design recommendations

6.2.1.1 Separation of ecosystem integrity and domestic use

Design recommendation: Ecosystem integrity and domestic use will be addressed separately.

Justification: This is consistent with the Department's philosophy of not regarding the ecosystem as a "user".

Implication: No specific attempt will be made to choose toxicity tests that will address both factors simultaneously.

6.2.1.2 Generic tests versus site specific tests

Design recommendation: A limited suite of generic toxicity tests will be chosen that will be applied nationwide.

Justification: Given the costs and likely logistical difficulties associated with nationwide capacity creation in respect of sampling and toxicity testing, allowing for significant site-specificity (and hence possibly a wide range of permitted toxicity tests) is considered likely to be inappropriately demanding. Choosing a few generic tests will also allow better quality control and hence standardisation.

Implication: Local site-specific conditions may occasionally be such that other toxicity tests might be more applicable. However, this will be a limitation of this national programme. It is nevertheless consistent with the broad "strategic" nature of national monitoring programmes. It is not the primary purpose of national programmes to facilitate reporting on specific local conditions. Its scope is defined as broad in both spatial and temporal scales.

6.2.1.3 Coverage of trophic levels

Design recommendation: For protection of ecosystem integrity, toxicity to three trophic levels, plants (algae in particular), invertebrates and fish will be monitored.

Justification: Monitoring only one trophic level can lead to potentially misleading results, in particular false negative results (*i.e.* reporting the resource is in an acceptable ecological category when it is actually not).

Implication: At least three fundamentally different tests will need to be chosen. This may be significantly demanding in respect of capacity creation on a nationwide basis.

6.2.1.4 Simultaneous measurement of lethal and sub-lethal toxicity

Design recommendation: Wherever possible and appropriate, toxicity tests will be chosen that are capable of measuring both lethal and sub-lethal toxicity simultaneously.

Justification: This can greatly reduce the costs of the required tests and limit the necessary capacity creation.

Implication: The number of tests is likely to be smaller than that necessary were different tests chosen for lethal and sub-lethal toxicity.

6.2.1.5 Water column versus biota or sediments

Design recommendation: Unfiltered samples of the water column will be monitored, not sediments or local biota.

Justification: (i) Representative samples of sediments are difficult to obtain. (ii) Assessment of sediment toxicity results is particularly complex. (iii) Inadequate capacity currently exists, and is unlikely to be easily created in the short-term, for such analyses and assessment.

Implications: (i) Concentrations of many, though not all, toxicants are likely to be lowest in the aqueous phase. This will create difficulties relating to detection limits. (However, using unfiltered samples may mitigate this problem to some extent.) Equivalently, the ability of both biota and sediments to accumulate many toxicants is a property that will now not be taken advantage of. (ii) Similarly, the loss of the time-averaging properties of both biota and sediments for many toxicants will mean that for these toxicants there is inherently a reduced ability to detect the effects of spikes of toxicants. (iii) More frequent monitoring may now be necessary (than would be the case had biota and/or sediments been monitored).

6.2.1.6 Active monitoring

Design recommendation: "Active" monitoring (involving equipment and organisms being left in the field for extended periods of time) will not be attempted.

Justification: Vandalism of monitoring equipment is a significant problem in South Africa. Although in some circumstances such monitoring is possible, for a programme of the magnitude of the NTMP loss or damage of equipment is of too great a concern. Adequate protection is unlikely to be feasible in all circumstances.

Implications: (i) Toxicity testing will necessarily need to be performed on samples taken in the field and transported to the nearest laboratory. This may create logistical problems if samples need to reach laboratories within 24 hours. (ii) The advantages of time averaging and accumulation in biota placed *in situ* are now not available. Therefore, one disadvantage of not using active monitoring is that the effects of toxicant peaks may not be detected. (iii) Effectively, this recommendation means that biomarkers of biota placed *in situ* will not be used in the NTMP. (iv) Similarly, so-called "passive samplers" (artificial *in situ* devices that simulate toxicant accumulation properties of biota) will not be used.

6.2.1.7 *Use of biota sampled in situ*

Design recommendation: Local indigenous (or even exotic) biota sampled on site will not be used.

Justification: (i) The practical difficulties of their capture and maintenance are too great for monitoring on a national scale. (ii) There is unlikely to be a single species that occurs in all our waters that will enable sufficiently standardised interpretation of results.

Implication: (i) Laboratory-bred organisms will need to be used in tests. Extrapolation of toxicity test results based on these organisms to likely effects on local indigenous biota may be difficult or at least involve uncertainty. (ii) This effectively precludes the use of biomarkers on locally sampled organisms.

6.2.1.8 Relative sensitivity of aquatic organisms and humans

Design recommendation: Aquatic organisms are assumed generally more sensitive to toxicants than humans.

Justification: This is generally indicated by toxicity data.

Implication: (i) Notwithstanding the above design recommendation not to assume that ecosystem integrity and domestic use should be addressed by the same tests, this recommendation will allow the toxicity data obtained for protection of ecosystem integrity to be assessed in terms of likely impacts on domestic use. Specifically, a toxicity problem detected in an aquatic organism can in general be regarded as a very sensitive test for humans. In essence, false positive results (in respect of humans) have an increased likelihood. Equivalently, if no toxicity is detected in an aquatic organism, the likelihood of a false negative result (in respect of humans) is very low. (ii) Not all possible toxic effects to humans are likely to be covered by a small set of aquatic tests.

6.2.1.9 Use of yeast test

Design recommendation: When the (desired) water use class is "Ideal" or when the (desired) ecological category is "Natural", the yeast test will be used (in addition to the chosen three trophic level tests).

Justification: This is a test that is specific to Endocrine Disrupting Compounds (EDCs). Since this broad group of compounds is of particular concern worldwide, and some of their effects will not necessarily be detected by the "trophic level" tests, this test is seen as an important supplementary test under conditions when the "best" (*i.e.* Ideal or Natural) water quality is desired.

Implication: This is an extra test requiring a further level of capacity creation.

6.2.1.10 Multi-context toxicity tests

Design recommendation: Whenever possible, and only when achieving the NTMP objectives is not compromised, toxicity tests should be chosen that either are currently being used in other contexts or are likely to be used in other contexts.

Justification: Given the significant costs of capacity creation relating to toxicity testing throughout the country, if tests can be used in more than one context then their overall cost-effectiveness increases.

Implications: As noted specifically in the design recommendation, there is a potential danger that when choosing tests, too great an emphasis may be tempted to be given to those that are, or will be, widely used at the expense of those that are more directly suited to the NTMP. This should be avoided. The NTMP should take priority.

6.2.2 Lethality versus sub-lethality

The following is the rationale behind the use of lethality and sub-lethality tests to detect whether the present state has been degraded from a Fair or Good category to Poor and from a Natural to a Fair or Good category, respectively.

- ***Long-term sub-lethality test for Natural to Fair/Good boundary***: The primary criterion is chosen to be **no toxicity of any kind**. This strictly means either lethality or sub-lethality, short-term or long-term. In effect this means any toxicity test will provide some information. However, **long-term sub-lethality tests** are chosen because it is assumed that these will be more sensitive tests (than lethality tests or short-term tests) and hence allow for more effective protection of ecosystem health.
- ***Long-term lethality test for Fair/Good to Poor boundary***: The primary criterion is chosen to be **no lethality (either short-term or long-term)**. However, **long-term lethality** tests are chosen again because it is assumed that these are likely to be more sensitive and more relevant to protection of ecosystem health than short-term lethality tests.

6.2.3 Results

6.2.3.1 Database

An initiative relating to guidelines for toxicity tests [see Relevant Initiatives in Background Chapter] provides a useful database upon which to impose the above design recommendations and produce a shortlist of appropriate tests. About 80 toxicity tests were classified as either being appropriate or not appropriate for each of the following criteria:

Table 6.1. Criteria for which toxicity tests were classified.

Screening / Definitive	Lethal
Active monitoring	Sub-lethal
Protective context: Ecosystem integrity	Short-term
Protective context: Domestic use	Long-term
Protective context: Agricultural – irrigation	Water type: Inland water resource
Protective context: Agricultural – stock watering	Water type: Estuarine
Protective context: Agricultural – aquaculture	Water type: Zone=Water body
Test organism: Fish	Water type: Zone=Sediment
Test organism: Amphibians	Water type: Zone=Groundwater
Test organism: Invertebrates	Water type: Fresh
Test organism: Plants	Water type: Brackish
Test organism: Microorganisms	
Test organism: Cellular or sub-cellular	
Test organism: Yeast	

This "inventory of tests" and their classifications exist in an Excel spreadsheet. This allows lists of tests to be created that satisfy multiple criteria. For example, it can list all tests that are (a) screening, (b) do not involve active monitoring, (c) are appropriate to protecting ecosystem integrity, (d) use fish as the test organism, (e) provide a measure of lethality, are (f) long-term tests and are appropriate to (g) the water body of a (h) fresh water of an (i) inland water resource.

6.2.3.2 Initial shortlists

Each of the contexts relating to the classification framework was considered in turn. As noted above, Natural ecological categories were assumed to require long-term sub-lethality toxicity tests while Fair/Good required long-term lethality tests. In order to minimise capacity requirements, the common tests in these two lists were extracted. In other words, tests were chosen that could measure both long-term sub-lethality and long-term lethality in the same experiment (for the same test organism). Such common lists were obtained for all cases except for plants. In the case of plants, only long-term sub-lethality tests were available.

Only screening tests were considered and active monitoring tests were excluded.

The following were the results:

Table 6.2. Initial shortlist for protecting ecosystem health (test organism: fish). These are appropriate for Natural and Fair/Good ecological categories and can determine long-term sub-lethality and long-term lethality in the same test.

Fish (zebra) development (semi-static)
Fish (zebra) development (static)
Fish (rainbow trout) development
Fish (fathead minnow) larval survival and growth
Fish (fathead minnow) embryo-larval survival and teratogenicity

Table 6.3. Initial shortlist for protecting ecosystem health (test organism: invertebrates). These are appropriate for Natural and Fair/Good ecological categories and can determine long-term sub-lethality and long-term lethality in the same test.

Daphnia pulex reproduction
Daphnia magna reproduction and survival
Ceriodaphnia reproduction and survival
Whole Daphnia cellular energy alloc. (lab. test)

Table 6.4. Initial shortlist for protecting ecosystem health (test organism: plants). These are appropriate for the Natural ecological category only (determines long-term sub-lethality only).

Duckweed growth inhibition
Algal 96-well microplate growth inhibition
Algal scintillation well growth inhibition
Algal 24-well microplate growth inhibition
Algal flask growth inhibition - chlorophyll measurement
Algal flask growth inhibition (various measurements)

For completeness, the same was done for domestic use. However, no restrictions were placed on test organism. The following was obtained:

Table 6.5. Initial shortlist for protecting human health (any test organism). These are appropriate for the Ideal and Tolerable/Acceptable water use classes and can determine long-term sub-lethality and long-term lethality in the same test.

Fish (zebra) development (semi-static)
Fish (zebra) development (static)
Frog teratogenicity
Ames Salmonella plate incorporation
Salmonella fluctuation (lab. method)
Salmonella fluctuation (Muta-chromoplate kit)
Umu mutagenicity

Recombinant yeast (hER)
Recombinant yeast (hAR)
Mammalian cell colony formation

6.2.3.3 Discussion of shortlists

Tables 6.2, 6.3 and 6.4 refer to protecting ecosystem health and use fish, invertebrate and plant test organisms respectively. This addresses the design recommendation that three trophic levels are tested.

Note that although individual shortlists were generated for sub-lethal tests (for the Natural ecological category) and lethal tests (for the Fair/Good ecological categories), only the tests common to both are reflected in the tables. That is, the same test can be used to determine both lethality and sub-lethality. This again addresses one of the design recommendations.

Domestic use is addressed as follows: A design recommendation was that protection of humans be assessed from two sources:

- The results of the tests chosen for protecting ecosystem health (*i.e.* chosen from Tables 6.2, 6.3 and 6.4), and
- The yeast test (to be used only when the water use class is Ideal).

In summary, it is Tables 6.2, 6.3 and 6.4 that need to be carefully examined and single tests from each chosen for the protection of ecosystem health in the NTMP. The single yeast test then needs to be added to this list for domestic use.

These considerations comprise the next step and must specifically address issues related to ease of monitoring (namely, costs of sampling, analysis and capacity creation).

6.2.4 Final recommendations

6.2.4.1 Fish toxicity test

Three tests were excluded from those in Table 6.2 for the following reasons:

- Fathead minnow tests: It is considered inadvisable to import these exotic fish for use on such a large scale.
- Rainbow trout test: This fish is associated with colder waters and is therefore considered inappropriate for use on a nationwide basis.

The two remaining tests were then ranked (1-3) on the basis of ease of monitoring. The following table shows the ranking used. Relative weights (1-3) have also been assigned to the five criteria.

Table 6.6. Ranking used for the fish tests.

		Ease of sampling	**Cost of sampling**	**Ease of analysis & assessment**	**Cost of analysis & assessment**	**Cost of capacity creation**
	Rank	1	1	3	3	2
Fish (zebra) development (semi-static)	**22**	2	2	2	2	3
Fish (zebra) development (static)	**18**	2	3	1	2	2

		1=Complex	1=High	1=Complex	1=High	1=High
		2=Intermediate	2=Medium	2=Intermediate	2=Medium	2=Medium
		3=Simple/routine	3=Low	3=Simple/routine	3=Low	3=Low

The recommended fish test is therefore: **Fish (zebra) development (semi-static)**.

6.2.4.2 Invertebrates

Of the four tests in Table 6.3 above, the daphnia magna test was excluded because this species is not currently used in South Africa and it is not prevalent in our waters.

The remaining tests were ranked on the basis of ease of monitoring, using the same relative weighting for the five criteria as above.

Table 6.7. Ranking used for the invertebrate tests.

		Ease of sampling	**Cost of sampling**	**Ease of analysis & assessment**	**Cost of analysis & assessment**	**Cost of capacity creation**
	Rank	1	1	3	3	2
Daphnia pulex reproduction	**21**	2	1	2	2	3
Ceriodaphnia reproduction and survival	**22**	2	2	2	2	3
Whole Daphnia cellular energy alloc. (lab. test)	**19**	2	3	3	1	1
		1=Complex	1=High	1=Complex	1=High	1=High
		2=Intermediate	2=Medium	2=Intermediate	2=Medium	2=Medium
		3=Simple/routine	3=Low	3=Simple/routine	3=Low	3=Low

Although the ceriodaphnia test is marginally better that the daphnia pulex test, it is considered best to choose the latter test because handling and maintenance of daphnia pulex is well established in South Africa.

The recommended invertebrate test is therefore: **Daphnia pulex reproduction**.

6.2.4.3 Plants

Of the six plant tests in Table 6.4, the 96-well test and the scintillation test were excluded because these are tests specifically developed for certain foreign countries and are, in any case, very similar to the 24-well test that is established in South Africa.

The remaining tests were ranked on the basis of ease of monitoring, using the same relative weighting for the five criteria as above.

Table 6.8. Ranking used for the plant tests.

		Ease of sampling	Cost of sampling	Ease of analysis & assessment	Cost of analysis & assessment	Cost of capacity creation
	Rank	1	1	3	3	2
Duckweed growth inhibition	**17**	2	1	2	2	1
Algal 24-well microplate growth inhibition	**27**	2	3	3	3	2
Algal flask growth inhibition - chlorophyll measurement	**18**	2	2	2	2	1
Algal flask growth inhibition (various measurements)	**18**	2	2	2	2	1
		1=Complex	1=High	1=Complex	1=High	1=High
		2=Intermediate	2=Medium	2=Intermediate	2=Medium	2=Medium
		3=Simple/routine	3=Low	3=Simple/routine	3=Low	3=Low

The recommended test is therefore: **Algal 24-well microplate growth inhibition**.

6.3 TOXICANTS

6.3.1 Final recommendations

The persistent organic pollutants (POPs) will comprise the initial "wish list" of toxicant monitoring variables for the NTMP. The following table lists the POPs. The PCBs (polychlorinated biphenyls), dioxins and furans comprise many compounds.

Table 6.6. The "persistent organic pollutants".

Chemical	Pesticide	Industrial chemical	Byproduct	Chemical Abstracts Substance No.
Aldrin	Yes			309-00-2
Chlordane	Yes			57-74-9
Dieldrin	Yes			60-57-1
Endrin	Yes			72-20-8
Heptachlor	Yes			76-44-8
Mirex	Yes			2385-85-5
Toxaphene	Yes			8001-35-2
DDT	Yes			50-29-3
Hexachlorobenzene	Yes	Yes	Yes	118-74-1
PCBs		Yes	Yes	
Dioxins			Yes	
Furans			Yes	

Given the well-established impacts of these compounds on ecosystems and human health (and their obvious international prominence), it is not considered particularly valuable to rank the POPs (among themselves) on the basis of their relative impact. Furthermore, if the NTMP is to be regarded as formally addressing (at least in part) the requirements of the Stockholm Convention,

all should probably be monitored irrespective of whether significant sources of them are known to exist in South Africa. The fact that they can be transported by atmospheric mechanisms from neighbouring countries (and those further away) also means that the non-existence of POP sources in South Africa is not necessarily an overriding criterion for their exclusion.

Accordingly, it is recommended that the choice of POPs included for the initialisation phase of the NTMP should be based entirely on criteria related to ease of monitoring. Three main criteria were recommended in Chapter 4:

- Sampling (ease and cost).
- Analysis and assessment (ease and cost).
- Capacity creation (cost).

Ease refers to the simplicity of the task and relates to the degree of expertise required. The cost of capacity creation refers to creating sufficient capacity nationwide (decentralised).

The following general statements can be made:

- Analytical methods do not exist in South Africa at this time for dioxins or furans. A screening test is being developed at Potchefstroom University. However, this is only likely to become available in mid-2005. It might be sometime after that (possibly years) that the test will be sufficiently well established and standardised to enable inclusion in the NTMP.
- A number of analytical laboratories exist that can analyse for the other POPs.
- The analytical method for PCBs and the pesticides involves an extraction then an analysis of the extract using gas chromatography and mass spectrometry (GC-MS). The PCBs and the pesticides may require two different extraction procedures. However, it may be possible to combine them.
- Once the extract has been obtained, a single GC-MS run provides the measurement for all the POP pesticides. If a separate extraction is required for the PCBs, then another GC-MS run will analyse for the PCBs.
- The cost of the analytical method is divided into two main parts: Extraction costs and analytical (GC-MS) costs.
- 1-litre glass sampling bottles are used for the PCBs and pesticides. They should be kept at 4°C and extracted as soon as possible after sampling (preferably within 24 hours).
- The extraction procedures are well documented but need a very reliable technician for consistent results.

From this it can be concluded that for the PCBs and pesticides:

- There is no difference in the sampling procedures (either in terms of ease or cost),
- There is no fundamental difference in the ease or cost of analysis and assessment,
- There is no fundamental difference in the costs of capacity creation.

The following is therefore recommended:

- Dioxins and furans should not be included in the NTMP at this time. This decision should be reviewed in future.
- The PCBs and pesticides should be included in the NTMP and no distinction (in respect of priority ranking) made between them at this time.

6.3.2 Guidelines

As noted in the Resource Classification Framework Chapter (Chapter 2), the two criteria for toxicity that determine the two boundary conditions for the resource classes also suggest the nature of the guidelines that should be used for toxicants. These are:

- No Observable Effect Concentration (NOEC).
- Maximum concentration that does not cause lethality (LC_0).

Values need to be obtained for these that take due consideration of the probabilities of false negative results and false positive results (see sub-section Consequences of Errors in Chapter 4: Criteria for Choosing Monitoring Variables).

6.4 DECISIONS TO BE REVISITED

All monitoring programmes must be revised from time to time to ensure that the original objectives remain valid and that they are being achieved. This should take place, at most, every five years but could be more frequent initially (every three years).

The following issues are recommended as important considerations in the first revision of the NTMP:

- The restriction of toxicity testing to samples of the water column only (*i.e.* excluding sediments). The advantages of sediment sampling should be carefully weighed against the disadvantages (including the costs of capacity creation).
- The inclusion of dioxins and furans if cost-effective decentralised analytical capacity can be created in South Africa.
- Inclusion of active monitoring.