Australasian Society for Ecotoxicology

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Ms Maureen Weeks
Committee Secretary
Senate Rural and Regional Affairs and Transport Committee
Department of the Senate
Parliament House
Canberra ACT 2600

30 November 2005

Dear Ms Weeks

Australasian Society for Ecotoxicology Submission on National Animal Welfare Bill 2005

We appreciate the opportunity to comment on the National Animal Welfare Bill 2005. The science of ecotoxicology is a multidisciplinary science involved in protecting the environment by understanding the fate, transport and effects of contaminants in the environment. This may involve field or laboratory assessment of chemicals and mixtures using a variety of methods, including field surveys and collections, laboratory toxicity tests with acute, sublethal and chronic endpoints, depending on the aim of the test, and bioaccumulation tests. The Australasian Society for Ecotoxicology, a member of FASTS, currently has about 200 members, mostly from Australia and New Zealand, but with others from drawn from various countries.

ASE members currently work under the *Australian Code of Practice for the Care and use of Animals for Scientific Purposes*. In most areas we have no difficulty in adhering to the Code, and support the need for such legislation, including the National Animal Welfare Bill 2005, in ensuring that animals are treated in a humane manner and that animal users are accountable, open and responsible.

ASE's concerns with some areas of the National Animal Welfare Bill are mainly about what we see as its unintended consequences for environmental protection, which may not be recognised by its proponents. These are detailed further in the attached submission but summarised here:

- Continued prohibition of the LD50 test or equivalent;
- The selection of cosmetics or sunscreens for prohibition;
- An all-encompassing definition of "animal" in Section 96 and conflicting definitions with Schedule 2;
- Inclusion of Malacostraca in the definition of "Animal" in Schedule 2; and
- The potentially severe consequences to the viability of small ecotoxicology facilities.

Fish and crustacean ecotoxicology testing, including on occasions lethality testing, is needed as part of a suite of tests to ensure protection of equivalent taxa in the environment. There is no other adequate surrogate for these tests that will provide sound robust results on which to base environment-related decisions. Inclusion of this broad area of crustaceans and effective banning of LC50 tests have very wide ramifications for protection of the water, soil and sediment environment in Australia against hazardous chemicals.

We trust that you will consider our attached submission.

Yours sincerely

John C Chapman
President, Australasian Society for Ecotoxicology

National Animal Welfare Bill 2005
Submission by the Australasian Society for Ecotoxicology

The science of ecotoxicology is a multidisciplinary science involved in protecting the environment by understanding the fate, transport and effects of contaminants in the environment, ie. in water, sediment, soil or air. This may involve field or laboratory assessment of chemicals and mixtures using a variety of methods, including field surveys and collections, laboratory toxicity tests with acute, sublethal and chronic endpoints, depending on the aim of the test, and bioaccumulation tests. The Australasian Society for Ecotoxicology currently has about 200 members, the majority of whom are in Australia and New Zealand, with others drawn from various countries across Asia and South Africa.

Although animal welfare legislation may vary across different states, in general, Australian research programs in ecotoxicology are currently subject to the National Code of Practice for the Care and use of Animals for Scientific Purposes*. In most areas our members would have no difficulty in adhering to the Code and being subject to the proposed Bill and support the need to ensure that animals are treated in a humane manner and that researchers are open, accountable and responsible.*

However ASE does have some concerns, mainly about what we assume are unintended consequences, and also some points of clarification. These are detailed further below but cover the following areas:

- Continued prohibition of the LD50 test or equivalent;
- The selection of cosmetics or sunscreens for prohibition;
- An all-encompassing definition of "animal" in Section 96;
- Conflicting definitions between Section 96 and Schedule 2; and
- Inclusion of Malacostraca in the definition of "Animal" in Schedule 2

While the requirement to report is not questioned, problems are encountered when prohibitions impact the work of ASE members and their efforts to provide scientific data to protect the environment.

General considerations: Animal welfare and environmental protection

Current animal ethics legislation in various states, and this proposed legislation, benefits society in ensuring that people dealing with animals, including researchers, are accountable, thoughtful and careful in their use of animals and that they consider their well-being.

Most animal welfare legislation in Australia is anthropomorphic (ie. based on attributing human characteristics to animals) and is based on human health or veterinary testing models, and not on environmental considerations with the need to address ecological links. Hence, ASE is concerned that the application of this legislation as it stands would not adequately consider the net benefits to the environment of toxicity testing and the serious impact on environmental protection if the appropriate tests are not done.

For instance, ecotoxicology is used to establish cause-effect relationships and hence contributes directly to sound management of the environment and protection of the species it contains. Pivotal to ASE's concern is the restriction placed on the use of fish in "LD50" tests, and the implications that extension into invertebrates has on obtaining information to support environmental protection measures. Despite progress in chronic testing, some LD50 testing on fish and aquatic invertebrates is essential to determine whether chemical wastes or complex mixtures are having, or likely to have, an adverse acute effect on these taxa in the wider environment. Furthermore, field sampling for aquatic ecosystem health assessment (such as the Sustainable Rivers Audit for the Murray-Darling Basin) would be tied up in this legislation. The ANZECC & ARMCANZ (2000) *Guidelines for Fresh and Marine Water Quality* relies on toxicity data for setting guideline values and for site-specific assessment under the risk-based decision scheme, and much of these data are by necessity acute data.

Testing of a small number of fish or invertebrates to protect ecosystems is quite different from that used to protect humans or veterinary animals. The latter extrapolate from just a few test species to protect, usually, one species. Ecosystem testing, on the other hand, involves extrapolating from a few species that best represent the different trophic levels in the environment, to complex ecosystems. For instance, the species used for ecological testing of water, sediment or soil are acting as surrogates for their own phyla or trophic levels in the respective ecosystems. It is internationally recommended in ecotoxicity that, for protecting the environment, a *suite* of tests should be conducted using organisms at different trophic levels (OECD 1992, 1995). Fish are the only test animals that can suitably protect fish, and likewise, crustaceans can only be used to protect crustaceans, and so on for other invertebrates. This principle is important in the regulation of hazardous chemicals to ensure safe levels of chemicals or complex mixtures in waters that do not cause degradation of the environment. There is no substitute for ecotoxicological data on a range of animals from different groups; and in cases where the exposure is high or the risk unknown, data from local organisms under local conditions are required.

The Animal Welfare Bill does not accord well with this concept of environmental biological testing for protecting ecosystems. The philosophical basis for the Bill seems to be similar to that of Dr Peter Singer (CET 1994). However, it is likely that the application of the Bill ignores Dr Singer's willingness to "allow forms of experimentation that, even though painful, were conducted on a small number of animals with the prospect of vast benefits to humans or animals " (Singer 1990 edition of *Animal Liberation,* p53).

The problem has been lack of recognition of environment protection priorities in the context of animal research and continued attempts to limit testing on fish, and now as proposed – crustaceans, in favour of alternatives that don't meet accepted scientific practice. This affects not only key areas for ASE in ecotoxicity testing and water monitoring but also the ability of state regulatory authorities to provide input into important State and National obligations in these areas. Some case studies are supplied in Appendix A.

Failure to use appropriate biological testing may be costly to the community, industry and/or to the environment, and may result in substantial high costs that will need to be borne by the Australian community.

Inclusion of Malacostraca

It is likely that cephalopods were included under the definition of "animal" in Schedule 2 because of their advanced neurological systems, and malacostracans to control mistreatment of large lobsters, crayfish and crabs in some areas of the restaurant trade. We believe that there are serious unintended environmental consequences of including smaller post-larval shrimps, prawns, isopods and amphipods within this legislation. To include malacostracans in the definition of "animal" would restrict the ASE, regulatory authorities and many other organisations in applying rapid testing for spills and incidents, in basic assessment of contaminated sediments, in routine monitoring, in deriving water quality guidelines, in implementing site-specific guidelines under the National Water Quality Management Strategy using direct toxicity assessment (ANZECC & ARMCANZ 2000) and a host of other programs such as SoE reporting, First National Assessment of River Health, Streamwatch, Sustainable Rivers Audit etc.

If the definition of 'animal' was extended to include these crustaceans, this would seriously affect the ability to protect the environment (water, sediment and soil ecosystems) from hazardous chemicals using invertebrate toxicity tests. It is not possible to find an adequate substitute for crustaceans as a surrogate for protecting their own phylum. In freshwater testing, microcrustaceans (Branchiopoda) are used (ie not included in this legislation) but there are many cases, particularly in marine waters, soil and sediment, where there is NO adequate substitute for prawns, isopods or amphipods. Furthermore, chronic tests with these Malacostaca can take up to 6 weeks and are fraught with uncertainties.

The assessment of contaminated sediments for the National (DEH 2002) ocean-dumping program relies on these few tests. The chemical complexity of sediments and interpretation is orders of magnitude more difficult than for water, due to the complex physio-chemical nature that affects toxicity. Biological assessment is the only reliable way to make decisions on complex contaminated sediment except in cases where chemical contamination is extremely high or very low. DEH (2002) recognises this in its internationally consistent tiered assessment. The only suitable whole-sediment test is with amphipods (ie. small burrowing crustaceans up to around 5 mm length). The acute lethal test takes 10 days and is the only test currently available for testing contaminated sediment nationally. The chronic test takes over 6 weeks, is less robust (ie. a greater potential for failure), and has not been established as a commercially available test.

The inclusion of any broad classes of invertebrates, such as crustaceans, under the definition of "animal" in the Animal Research Act also has other wide-ranging and significant ramifications for ASE and environmental protection in general. Any sampling of water or sediment from aquatic systems for scientific purposes (eg experiments, environmental monitoring and ecological surveys) will affect some individual crustaceans. The types of activities such changes to the Act could affect include:

- Monitoring for toxic algae, bacteria and viruses in waters for the protection of human health and Environmental Impact Statements;
- Monitoring of water quality for local government's state of the environment reporting;
- State participation in the First National Assessment of River Health (Monitoring River Health Initiative) that uses standardised methodology to protect aquatic systems;
- The national ocean disposal program (DEH 2002) involving sediment toxicity testing;
- Environmental monitoring as part of state EPA (or equivalent) licence requirements;
- Aspects of rapid response to spills and incidents, including oil spills under the National Oil Spill Contingency Plan (especially quality control aspects);
- ASE's involvement in site-specific studies to develop more rigorous environmental criteria (eg. ANZECC & ARMCANZ 2000) for the protection of the environment; and
- The prosecution of polluters under state legislation, which usually requires laboratory or field investigations over a short time to establish actual or potential environmental harm.

In summary, crustacean testing, including lethality testing is needed to ensure protection of crustaceans and other invertebrates in the environment. Inclusion of this broad area of crustaceans has very wide ramifications for environmental protection in Australia.

ASE recognises that community concerns about crabs and lobsters in restaurant situations may be valid. However, any extension of the Act to cover crustaceans generally would have extremely serious consequences for protection of water, sediment and soil ecosystems against hazardous chemicals. There is no other adequate surrogate for these tests that will provide sound robust results on which to base environment-related decisions.

If the Bill were limited to *"Decapoda of the clade Reptantia (ie. lobsters, crabs and crayfish"*, this should satisfy most of the handling concerns and free up shrimps (which are also decapods) as well as isopods and amphipods for environmental protection measures. That will however not satisfy protection concerns for crayfish and crabs in freshwaters, which can be the dominant macrocrustaceans in freshwaters.

All-encompassing definition of "animal" in Section 96

96 Definitions

In this part:

***Animal** means an invertebrate or vertebrate animal other than a human being*

This definition is <u>extremely</u> broad and will generate a huge amount of needless paperwork, without serving society in any useful way. This would severely limit work with an enormous range of invertebrates, such as ants, flies, cladocerans, amphipods, brine shrimp and a host of larval forms of organisms. This broad range would most likely result in non-compliance, which defeats the

purpose of the Bill. It is important to get the animal definition right if the legislation is to maintain credibility.

Any further restrictions on the definition of animal would also have significant ramifications on the application of the revised ANZECC & ARMCANZ water quality guidelines for Australia and New Zealand. Direct toxicity assessment, which involves aquatic "animals" in site-specific assessments, is integral to the final decision on whether a potentially high-risk chemical concentration presents an actual risk to the environment.

In addition, routine bio-physical monitoring activities would be affected significantly. This type of monitoring relies on sampling various volumes of water and then analysing that water for a range of physical, chemical and biological properties. Most water samples are likely to contain microscopic crustaceans ("zooplankton"), which are not the aim of the investigation but which will be killed during preparation of samples for analysis. Biological assessment of river health relies on sampling of animals. Just about any form of biological assessment would be captured by the new Bill. This introduces a double standard, as what would be legal for the many recreational fishers, would be illegal for the relatively small number of investigators with the aim of protecting the environment.

This has major ramifications in areas involving not only government agencies but also environmental consultants, local government and community groups. State participation in national monitoring programs of waterways, ecological surveys, state of the environment reporting, Streamwatch programs, sediment assessment and the full assessment of environmental harm for prosecutions would be either severely restricted or prevented.

Conflicting definitions of "Animal" in Section 96 and Schedule 2

96 Definitions

In this part:

***Animal** means an invertebrate or vertebrate animal other than a human being*

Schedule 2 – Definitions

In this Act, unless the contrary intention appears:

***animal** is any of the following:*

- *(a) a live member of a vertebrate animal taxon;*
- *(b) if it is in the last half of gestation or development a live pre-natal or pre-hatched creature that is:*
  - *(i) a mammalian or reptilian foetus;*
  - *(ii) an avian, mammalian or reptilian pre-hatched young;*
- *(c) a live marsupial young;*
- *(d) a live invertebrate creature of a species, or a stage of the life cycle of a species, from the class Cephalopoda or Malacostraca prescribed under a regulation for this paragraph.*

However, a human being or human foetus is not an animal. To remove any doubt, it is declared that the following are not animals:

- *(e) the eggs, spat or spawn of a fish;*
- *(f) a pre-natal, larval or pre-hatched creature, other than a creature mentioned in paragraph (b);*
- *(g) another immature form of a creature, other than a creature mentioned in paragraph (b).*

The two definitions do not accord and it is unclear from the draft legislation which one prevails. If the former general definition prevails, this is too broad to be practical; if the latter, this still causes problems with the generality of "Malacostraca", as noted earlier.

We have very grave concerns over the seemingly innocuous wording in clause "d" "*prescribed under a regulation for this paragraph".* This seems to be lifted from the Queensland legislation, and where it may be legal to continue testing in the absence of a regulation under the QLD Act, but

this may not be the case for a Federal Act. Our major concern is that the reference to a regulation is a potential quick mechanism to add malacostracans as "Animals" at a later date without requiring an amendment or public consultation.

Continued prohibition of the LD50 test or equivalent

81 Use for certain scientific purposes unlawful

A person must not, without the Authority's written approval:

(b) conduct the test commonly known as the classical LD50 test, or a similar test; or

(c) …….

ASE considers that, for environmental testing, the benefits to the community of using fish and crustacean testing for environmental protection from hazardous chemicals, including lethality testing where appropriate, are substantial. This is not to imply that there should be unlimited LC50 testing (note that LD50 and LC50 are used interchangeably here; LC50 is the concentration that causes death to 50% of test animals). ASE members are involved in continual development of tests that provide robust alternatives to LC50 testing but in some area this is not yet achievable, nor may it ever be. Appendix B covers some views of ASE on the drive to alternative testing in the context of environmental protection.

These net community benefits include knowledge on what is affecting our waterways, soils, sediments, animals and ecosystems from emissions of chemicals that can have significant impacts on both animal and human health. Such knowledge has the power to change behaviours and inform both industry and the community on better ways to protect the environment.

The prohibition of "LD50 testing" (which may make sense from human health or veterinary testing viewpoints, but not from environmental viewpoint), is not matched in most countries throughout the world (based on an ASE survey of researchers from around 20 countries; see Appendix C). The proposed ban on fish and malacostracan LC50 testing in the Bill is out of step with legislation in other countries and stands opposed to international recommendations and approaches in managing hazardous chemicals (OECD 1992,1995).

The application of the Bill has the potential to drive environmental work offshore, despite the fact that research aims to ensure that ecologically sustainable development is maintained, to derive safe levels of chemicals or complex mixtures in waters, soil or sediment that will neither cause degradation of the environment nor cause unnecessarily onerous burdens on industry. Failure to use appropriate biological testing will be costly either to industry and the community or to the environment, a cost that the community will pay in any event. At present, the issues of public benefit of testing animals for protecting the environment, as well as the costs if there is no testing, are not adequately weighed alongside the benefits of imposition of the Act.

Some LC50 testing on fish and invertebrates is essential to determine if chemical wastes or complex mixtures are having an adverse acute effect on these taxa in the wider environment. Acceptance of ecotoxicity test data in Environment Courts requires NATA certification, which in turn requires reference toxicant testing with LC50 measurements. Aquatic LC50 testing for environmental protection should only be subject to scrutiny by local animal care and ethics committees and should not be subject to such wide-ranging prohibition.

ASE understands that the basis for restrictions on LC50 testing with fish and potentially some invertebrates, is their capacity to feel pain. The issue with fish is still very controversial (see the Norwegian website http://oslovet.veths.no/dokument.aspx?dokument=175 and scientific references cited within; (eg Rose et al 2002) and it is even more uncertain when applied to small crustaceans used for the relatively few ecotoxicology tests.

The selection of cosmetics or sunscreens for prohibition

81 Use for certain scientific purposes unlawful

A person must not, without the Authority's written approval:

(c) use an animal for a scientific purpose if the use involves testing:

(i) a cosmetic; or

(ii) a sunscreen product; or

(iii) *an ingredient of a cosmetic or sunscreen product*

It is understood that the aim of this clause is to eliminate trivial testing using animals. Again the focus is on testing from human health viewpoint, and does not consider the very different approach of environmental testing, where the environmental effects of these products are largely unknown. Recent monitoring in the USA has shown that such products are prevalent in waterways and it is important to better understand their effects so as to provide effective and practical environmental management.

Commonwealth funding of research:

117 Prohibition on research funding

(1) The Commonwealth must not provide funding to any organisation that uses animals for research & development

Unless the Minister is satisfied that the organisation's use of animals is consistent with the objects of this Act and does not contravene any code of practice established under the regulations

It is understandable that this clause is included but if there is no provision for legitimate exemption for certain activities, such as environmental testing, then it has the potential to cut Commonwealth funding for much nationally necessary ecotoxicological work.

Additional concerns for commercial ecotoxicity test contractors

Ecotoxicology testing facilities in Australia are small businesses that run on a shoe-string but provide an important service to industry and government. There are three major implications for such laboratories:

1) '*Supply Unit licenses*'- The bill, if passed in its current form, will require all facilities that supply animals for any scientific purpose to be licensed as a supply unit. The degree to which this will affect small ecotoxicology ("ecotox") laboratories largely depends on the definitions of an animal in the bill, which could be all animals apart for humans (as per Section 96) or vertebrates plus malocostraca and cephalopods (as per conflicting definition in Schedule 2).

This is a significant problem, in that most hatcheries (or other laboratories) that supply ecotox laboratories with prawns, amphipods etc do so if convenient to them (ie. they are often doing us a favour in supplying us). The Bill provides a marked dis-incentive for a hatchery or laboratory to jump through all the hoops of obtaining a supply unit licence to be able to supply ecotox labs on an occasional basis. Further, using animals by any of us from a supplier without a license would be in breach of the Act.

2) Financial effects on businesses based on ecotox testing: There are very few specialist commercial ecotox testing laboratories in Australia. The banning on LD50 or similar (which we take to be LC50s) would have two main effects: The revenue stream may be reduced by anywhere between 25 and 90%, depending on the definitions of "animal". Most of the tests commissioned on a commercial basis are either LC50 tests or similar (ie EC50s, dose-response short term direct toxicity assessments), and the end result may be a collapse in the revenue stream, the business and employment. Even a 25% revenue stream would result in severe cuts to laboratory staffing and even result in closure of businesses.

3) The third effect is that if these small commercial ecotox providers disappear, then we may be in a situation where there will be no-one to provide ecotox services to fulfill site-specific ANZECC & ARMCANZ or Ocean Dumping guidelines, or even toxicity testing requirements for the chemical assessment NICNAS and APVMA chemicals for registration purposes. This would mean that the

regulatory priorities in this field would be stifled, and Australia would be forced to rely on either inappropriate overseas data or no data at all.

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APPENDIX A: Case studies of toxicity tests protecting the environment

The demonstration of environmental harm is a key tenet in prosecutions in much state environmental legislation. Ecotoxicity tests can provide an important link between chemical levels and effects. In many cases, ecotoxicity data have assisted agencies and the court to set penalties appropriate to the harm caused or likely to be caused. Licensees have been required to undertake ecotoxicity testing as a condition of licence in cases where the effluents were particularly complex.

Chemical spills in Sydney Creeks

Ecotoxicity tests are used to for decisions whenever there is a chemical spill, fish kill or incident involving a chemical. Ecotoxicity tests can be employed to assess the likely hazard, determine the cause and provide information on which to base decisions in the field.

One typical example of is a recent fish kill in Dents Creek. After an unidentified chemical spill into the creek caused a fish kill, the creek was dammed to minimise downstream effects and the material was pumped out. Testing with caged fish and microcrustaceans showed that toxicity was still present in water from upstream flows, and the dam was kept in place until ecotoxicity tests confirmed that toxicity from upstream was negligible. The location of the discharge was traced using crustacean tests in the field on samples collected from different stormwater drains. A major incident in Prospect Creek in 2002 involved a pesticide spill accident from an industrial area, resulting in major fish kills. The spatial extent of the toxic plume was and the degree of dilution were traced with toxicity tests and the results of these fed back into operational decisions. During this process, an additional source of toxicity was discovered, resulting in a major audit for Prospect Creek involving several councils and the DEC.

In another incident, an unknown toxicant in the STP for a rural city caused the plant to be shut down for nearly a week until fish tests give clearance. Chemical tests could not identify the toxicant and there were no adverse effects on crustaceans.

***In-situ* on site tests for industrial effluent**

A major Australian industry applied to reduce its air emissions by applying a fine spray mist through the air emission and discharging the wastewater continuously into a harbour. This would have cost around $5 million, instead of around $18 million for the full treatment to discharge clean water. At the request of a regulatory authority, in-line fish tests on the discharging wastewater were set up. After 6 months, analysis of the fish tissue showed lesions, resulting in a willingness by the company to spend the extra $13 million.

Acid sulfate water leachate

Tests with Australian bass *Macquaria novemaculeata* established that acid sulfate waters mixed with estuarine water results in significant mortality of fish and oyster larvae. These data ensured successful injunctions and prosecutions associated with coastal urban developments adjacent to estuaries and provided strong background support for state government restrictions on canal development in estuaries adjacent to acid sulfate soils.

APPENDIX B: Alternative Testing options for environmental testing

Animal ethics legislation relating to research usually incorporates the "3-Rs"; Refinement, Reduction, Replacement. This is admirable in theory and, for the human health or veterinary field, in practice. However, in the environmental area, "replacement" is often not a sensible option. Alternative testing is usually not possible when testing for the effects of hazardous chemicals in aquatic environments. We emphasise that use of *in vitro* tests, modelling techniques or other types of animals can not adequately or reliably produce the results necessary to protect both animals and the environment from future effects of chemical pollution incidents. Ecotoxicity testing is the key to this process, and lethality testing is essential for evaluating situations where mortality is the indicator of environmental concern, ie. such as investigating fish kills, in order to determine remediation and recovery measures.

This drive to alternatives under the NSW Act has previously led to refusal of NATA accreditation for fish lethality tests in 1998, because attempts to accommodate this view were not acceptable to the NATA assessors on scientific grounds. The acceptance of ecotoxicity test data in state Environment Courts requires NATA certification, which in turn requires reference toxicant testing with LC50 measurements. In many cases, there are no alternatives other than to use fish to test what is harmful to both fish and the environment. This is not recognised by the current NSW Act or by the draft National Bill. If invertebrates are caught up in the Bill, this would cause added complications.

What is not considered is whether alternative tests such as use of *in vitro* tests, modelling or testing animals from other phyla (eg bacteria) are appropriate or scientifically acceptable. Most of these "alternatives" are not appropriate for protecting fish or crustaceans from effects of hazardous chemicals in aquatic environments, singly or in complex mixtures. The ANZECC & ARMCANZ (2000) water quality guidelines incorporate the latest approaches in risk assessment in applying guidelines to specific sites, and this requires some assessment with local species under local conditions. The test endpoints must be tailored to the specific situation, and lethality testing is appropriate for evaluating situations where mortality is the endpoint of environmental concern, such as for emergency response, pesticides issues, fish kills etc. Most "alternative" techniques are only accepted internationally as part of a suite of toxicity tests, not as replacements.

Physico-chemical measurements

ANZECC & ARMCANZ (2000) recognise that physico-chemical measurements (eg. pH, conductivity, dissolved oxygen, turbidity, etc.) and chemical measurements alone are inadequate to protect the environment. This problem is exacerbated in cases where the toxicants appear in complex mixtures and biological assessment is the only appropriate measure. There are a number of chemicals, particularly some pesticides, where biological effects occur at concentrations that cannot be practically measured chemically at all.

Microtox ® and other bacterial bioluminescence assays

This involves measurement of changes in light output from a particular strain of bacterium. The endpoint bears no relevance to any ecological function that would indicate threatening conditions for any species in the aquatic environment. Its test conditions are artificially saline, precluding any reasonable assessment of site-specific water quality conditions. Its sensitivity to a number of compounds is poor and effects from pesticides, for instance, occur in the milligram per litre range, whereas fish and crustaceans are affected in the low µg/L range.

Sub-mitochondrial tests

These bioassays use sub-mitochondrial electron transport particles (ETP) to evaluate chemical toxicity. Like Microtox ®, these bioassays are conducted under artificial media conditions, which do not reflect normal variations in water quality. The ETP test can not take into account normal species differences, nor can they readily reflect bioavailability considerations and charges in toxicity with different water types.

Structure – activity relationships (SARs)

SARs are useful where there are no data but are not applicable to assessing site-specific field situations. SAR modellers agree that their proper use is for extending limited data sets and for this reason neither OECD (1992) nor US EPA accept SARs on their own for development of water quality criteria.

Chronic sublethal testing

ASE member groups are continually and successfully developing chronic tests to complement acute testing. Several issues need to be addressed:

- Not all chronic endpoints are ecologically-relevant (OECD, 1992). At the current stage of knowledge, most biomarker endpoints can not be related to potential changes in ecosystems. Such effects may be reversible responses to toxicants that may occur at concentrations quite unrelated to ecologically-relevant effects such as mortality, immobilisation, reproduction, growth and certain behaviours.
- Chronic endpoints may be irrelevant for acute exposures. Modes of action for chronic toxicity may be different than those for acute toxicity (Baird *et al.* 1990).
- Chronic tests usually take longer than acute. In emergency response and prosecution cases, where action is required, this is a problem. For sediment testing, chronic tests can take up to 6 weeks.
- Chronic endpoints can often be less robust than acute, resulting in wider error bars, greater chance of rejection of tests and more difficulties in interpretation.

Algal testing

Algal tests have been developed in NSW (Stauber *et al.*, 1996) and are very useful in a suite of toxicity tests. To some chemicals, particularly some herbicides and metals, they can be very sensitive. However to others, algae can be extremely insensitive (eg. to the pesticides chlorpyrifos, endosulfan). This further emphasises the completely different physiology and biochemistry of plants and the need to perform tests on a suite of species when using such tests for environmental protection.

In summary, ASE member groups are continually refining the range of ecotoxity tests with an emphasis on developing robust chronic tests in order to minimise the number of tests using lethality as the endpoint. In addition, alternative systems such as Microtox ®, passive samplers filled with solvent to mimic fish gills and SARs are all used and have their rightful place in a suite of tests used. Chronic tests and the above “alternatives” are not relevant to each and every situation, and the Australian & new Zealand Guidelines recognise that site-specific evaluation of each situation is important. Their use in Australia has gone some way in reducing numbers of animals used but it will not be possible to use these as outright replacements for aquatic animal testing for protection of ecosystems.

I wish to make the following recommendations:

1. In the interests of weighing the net environmental benefit, all ecotoxicology experiments for protection of the environment, including lethality tests that use fish and crustaceans, should be exempt under the Act for the requirement for Ministerial or ARRP approval. All such research should still be submitted to ACEC consideration.

2. The definition of animal in the Act should not be extended to include crustaceans.

APPENDIX C: Replies Received Regarding the Use of Fish and Crustaceans in Ecotoxicology Tests - July 1999

Brazil

In Brazil we do not have any legislation that requires official approval before the use of fish or any other vertebrates in toxicity tests. This is true for both federal and state legislation. Fish & other vertebrates and invertebrates can be used in toxicity tests in Brazil. Several universities,

independents laboratories and states environment agencies use toxicity test. The only one thing that is controlled is the use of exotic species, in order to prevent the dissemination on the natural environment.

Chile
This is an area in which, as far as I know, and after many years doing ecotoxicology research, **there are no regulations whatsoever in Chile**, either by Government agencies or University panels. No permits are required to do toxicity tests on fish and crustaceans. My guess is that this reflects the situation in all of Latin America.

Denmark
As far as I know **there are no such limitations (on the use of fish and crustaceans) in Denmark or the EU for that matter**. At the universities students do ecotoxicology projects with fish and all sorts of invertebrates without any sort of permit required.

At the legislative level there is a legal requirement that toxicity tests be conducted with the so-called base set (a fish, Daphnia (a crustacean), algae) for all industrial chemicals and pesticides. For pesticide risk assessment additional tests with fish and other non-target invertebrates (insects, benthic crustaceans, molluscs) may be required as part of a refined risk assessment.

Europe
In Europe, several pieces of legislation, and in particular the legislation for the notification of new chemical substances, require animal testing to generate a database on the chemicals to be put on the market. However, at the same time, there are efforts to decreased the use of test animals and a European Bureau (ECVAM - European Centre for the Validation of Alternative Methods) on the topic has been set up at the European Joint Research Centre.

France and the EU
In Europe, the ecotoxicological tests on fish and shellfish are obligatory before the placing a new chemical substance or product on the community market. The necessary tests are standardised and described in European directives 67/548/EEC and 92/32/EEC.

In short, these directives establish that the laboratory and the personnel charged to carry out tests on animals must be equipped with governmental authorisations. These authorisations are granted for the laboratories which observe the rules of Good Laboratory Practice, and to the personnel which have followed a specific national training in particular with regard to the animal handling ; his formation is validated by a diploma.

Directive 86/609/EEC applies to all living non-human vertebrates, including the larval forms autonomous and/or capable of reproduction, but not the fetal or embryonic forms.

In practice, in France the fish are not taken into account and no procedure or authorisation is necessary to carry out tests on these animals. With regard to shellfish, no European directives takes them into account.

Germany
First reply
The situation for animal testing in Germany is briefly as follows:
- approval is needed for tests with vertebrates including fish
- tests with invertebrates have to be announced to authorities beforehand

In both cases, a person with a relevant education (veterinary, toxicology or zoology) must officially be responsible for animal testing at the institution of interest and this person be announced to authorities. For each particular experiment, another person responsible for the experiment (and having a similar qualification) has to be selected and reported to authorities. Protocols for animal

maintenance before tests and handling during tests have to include precautions to avoid suffering of animals etc. Authorities also sometimes visit labs without announcement and check lab practice.
The approval procedure is controlled by local (province) authorities and may slightly differ among provinces. In the forms, the following aspects are considered (among others):

- justification of the need for the research
- how severe is the anticipated pain / suffering of animals during the experiment (will be ranked depending on the type of experiment)
- attempts to limit the number of animals used

From my experience and from what other people in environmental toxicology tell I have the impression that in Germany the control by authorities is quite strict but it is possible to do animal testing.

Second answer from Germany
We use different kind of fish (*P. pimephales, Danio rerio, Leuciscus idus, Oncorhynchus mykiss*) and one crustacean, namely Daphnia magna. Some of the fish are bred inhouse. We need an approval for that, but to my experience this is not a major problem. No approval is needed for the Daphnia cultures.

Since the tests we do serve for risk assessment (in almost all cases required by law) and not basic research, we apply for a general approval every year. This application is accompanied with a list of all animals (including the mammals of our classical toxicological tests), the envisaged number of animals and the type of tests intended. Usually this application is approved soon after the authorities get it. It then lasts for a year. At the end of this year the authorities get the list of all tests, which were really performed.

If it were for basic research (as do our pharmacologists with their mammalian test animals), we would have to apply for each test separately.

Third answer from Germany
In Germany there is a distinction between tests, which are required by law (e.g. notification of chemicals, registration of pesticides) and those tests that are not required by law (e.g. research).

1) Tests on vertebrates which are required by law need only a notification. This notification is not time limited.
2) Tests on Cephalopda or Decapoda need in any case only a notification (not time limited).
3) Tests on vertebrates which are not required by law need formal approval by an ethic commission. This needs normally 3-4 months to get approval. The chance of getting an approval depends on the justification and on some basic requirements, you have to fullfill. The approval is given for the duration of the tests but in the maximum for 3 years. Then you have to write a new application.
4) Tests on invertebrates need neither notification nor approval (with exception of Cephalopoda and Decapoda). This is new, in former times we had to notify also tests on invertebrates. But since 1998, the new law was issued.

Hong Kong
In short, we have restrictions only on the use of mammals (i.e. rats and mice). Permits will be issued as long as one can demonstrate the need to use such animals, and all necessary steps are taken to minimize any “inhumane treatment of the animals”.

Indonesia
It seems to me that the situation you are facing in Australia is quite different to what I have in Indonesia. Here there is no existing regulation on the use of test animals, except those included in

the CITES list. Some issues, however, have been raised related to the use of primates in medical experiments.

Ireland
I hope my responses will allow you to put a good case forward to maintain this type of testing in Australia.

Toxicity tests are used widely in Ireland to regulate and monitor the impact of industry on aquatic environments. In particular, both the Irish EPA and some of the regulatory authorities who control the smaller industries require industry to monitor effluent discharges for toxicity. Also there may be a requirement to undertake ambient river water toxicity monitoring and also testing on wastes for landfilling and also leachate from landfills. We usually ask for acute toxicity testing to be carried out against four trophic levels (screen test) - bacteria, plant/algae, crustacean, fish. As a follow on, compliance monitoring is usually done on the 2 most sensitive species.

To date in Ireland we (Irish EPA) have control of approx. 550 industries and 150 of these would have a requirement for toxicity testing. This does not include toxicity monitoring on wastes, landfill leachates etc.

Currently there are no restrictions controlling the use of any of the species in toxicity tests. However, there is likely to be some control brought forward in the near future (similar to Germany).

The only permits required are for the catching of the test species and these permits are issued by the Dept of Marine in Ireland.

Israel
Fish and crustaceans can be used for ecotoxicity testing in Israel. There are no restrictions on their uses, and no permits are required. Please excuse my frankness, but I find the intention to expand this restriction to crustaceans ridiculous.

Italy
First reply
As far as I know we do not have any restriction in the use of fish or crustaceans for toxicological tests. In the European Union legislation on dangerous chemicals (6 amendments and following) compulsory toxicity tests on fish and crustaceans are required before new chemicals can be put into the market.

Second reply
At present, in Italy as well as in other Member States of the European Union, there are no restrictions on fish and invertebrate testing. There are some ethical constraints for terrestrial vertebrates, in particular warm blooded (birds and mammals).

In practice, by submitting a research project for founding you must declare that animal testing will be reduced as much as possible and will be used only if there are no suitable alternatives. In reality, this statement is more formal than substantial. On the other hand, all European Directives for testing new and existing chemicals include animal testing (vertebrates and invertebrates). For the registration of a new pesticide in Europe (as well as in USA) the industry must provide a big dossier on toxicological and ecotoxicological testing (invertebrates, fish, birds, mammals). Therefore, if animal testing is required for official regulations, obviously it must be accepted for research. How do you made toxicological and ecotoxicological tests on new chemicals in Australia?

Malaysia

In Malaysia we don't have such a strict regulation or legislation pertaining the use of animals for research. We are more relax, and free to choose the subject. However, it is very much depending on the species and area. We are required to write a full research proposal, nevertheless, the authority is always in support, as long as the number is reasonable, and literally, one should be able to work on any fish or vertebrate species without any specific licence.

Mexico

In Mexico there are not prohibitions about animal uses related to lab work or research. Fishes, and rodents as well as all invertebrates, not in-danger, can be used with not restriction for experimentation.

New Zealand

First reply

Fish and other crustaceans are used in toxicity testing in New Zealand.

According to the 'National Guidelines for Institutional Animal Ethics Committees' the definition of an animal for the purposes of the Animal Protection Act 1960 is:

(a) any horse, cattle, sheep etc etc ....
(b) any bird
(c) any marine mammal
(d) any vertebrate animal that is kept in a state of captivity or is dependent upon man for its care and sustenance
(e) any animal of a species that is declared by the minister, by notice in the Gazette, to be a species of animal for the purposes of this Act.

So fish in the lab fit under category (d)

A new bill is due to be tabled in Oct 1999, which is going to further define fish into the definition ie so even wild sampled fish will be included (I dont really know how, except it wont be under section 3e).

So... inverts seem OK for the moment - last time I spoke to a committee rep about it - they reassured me that inverts (at least soil inverts) will be OK ... but we did not specifically discuss crustaceans.

Second reply

Our Animal Ethics responsibilities are set out under the Animal Protection (Codes of Ethical Conduct) Regulations 1987 and the Animals Protection Act 1960. This is about to go under review.

We have a formally constituted committee for NIWA and a National Protocol which has been approved by the Minister. This requires us to keep track of numbers of vertebrates and is legislation specific.

Our Animal Ethic Committee has: NIWA, Chair (Ops Mger); NIWA Sec; Public Vet; Welfare Group, SPCA; Independent Vet; Independent Person, Regional Council. Guidlines are available for their decision making.

For toxicity all studies are approved by the Committee. We have a blanket approval for vertebrate toxicity testing on all chemicals which are currently released to the environment. Any new chemicals need special approvals. We keep track of the species and numbers used. All must follow approved protocols.

There are no restrictions on crustaceans or other invertebrates (eg shellfish).

We have a rapid turn around for approvals for studies providing the case is well specified.

Good luck with your submissions. Some sense needs to be brought in here as these substances are being released in your environment!

Russia
There are no strong restrictions for usage of animal for toxicological research or for biomedical research.
There are no strong legislation basement on regulation of the animal usage for research aims and scientific ethics too in Russia.
There are also no restrictions to use human tissues.

Spain
First reply
Fish, vertebrates and crustaceans can be used in toxicity tests. There are no restrictions or permits required to conduct this type of research.

Second reply
As far as I know there is no legal restriction to their use. As you know, Spain is a member of the European Union (EU) and the legislation approved by the EU has to be also implemented in my country.

South Africa
We do not have any restrictions on the use of fish or any invertebrates. This applies to all universities and institutions that carry out ecotoxicology work. Each university is obliged to have an ethics committee to ensure that we adhere to broad principles set by the SPCA (Society for the Prevention of Cruelty to Animals).
I do know that the medical reseach laboratories have some legislation governing their use of mammals in research.

Sweden
First reply
All animals can be used for scientific experiments, including toxicity tests, except those of course which are on the extinct list.

There are no particular restrictions other than you have to get approval from the Ethical Committee for Animal Research (our IACUC).

As I mentioned above literally all scientific experiments that includes animals have to be passed by an approved by the ethics committee. The committee meets once a month and have about 30 applications each month to deal with. If the scientific purpose and the harm inflicted on the animals is reasonable, it is pretty easy to get an approval. From this to harmful experiments it is increasingly difficult to get approval.

Second reply
My general feeling is that animal testing is mainly done for other purposes than toxicity testing on chemical substances - the Swedish chemical industry is rather small and the testing on imported chemicals is done by the mother companies in US, UK, France etc. Most of the testing in Sweden is done by research laboratories, the pharmaceutical industry and regarding ecotox it is dominated by effluent testing. There is, however, a strong ambition to avoid unnecessary animals testing and KemI has also been active in the OECD work on alternatives to animals testing (within the OECD Test Guidelines Programme).

Third reply
Sweden has a system for ethical review of the use of animals for scientific purposes. These reviews are required in the case of live animals belonging to the following classes: mammals,

birds, reptiles, amphibians, fish and cyclostomes. The use of crustaceans is not regulated by law. Projects can be approved for a maximum of three years.

Switzerland
Vertebrates, including fish and frogs, are protected under the law in all EU member states and in Switzerland, i.e. a special application is required before toxicity testing may be conducted.

There is no regulation for invertebrates in place and there is also no related discussion ongoing in any of the EU member states.

Note that the Swiss procedure/law is one of the most restrictive laws in the world.

The Netherlands
First reply
In the Netherlands some kind of approval is necessary to use organisms in toxicity tests but only for rats, mice, monkeys etc., not for fish and crustaceans. People working with organisms that are incorporated in the law of test-animal use, also need some kind of certification that they are able to work with them and are registered. This law exists already for a long time, but I never heard any rumours to include fish and invertebrates into it.

Second reply
- I know that on vertebrates there are restrictions, not for invertebrates.
- When conducting testing for notification (required by legislation) one always gets permission. For more fundamental research on toxicity tests to fish, forms have to be filled in. To my knowledge it's easy however to get permission for fish tests. However, not for mammals of course!

Third reply
The Dutch animal legislation stems from 1996. Test animals that are considered by this legislation are all vertebrate species (except fish eggs and larvae) and announced invertebrate species. There are no invertebrates announced yet, and it is not expected that this will change.

Animal tests are subject to "prior" consent from an expert commission authorised by the Minister for Public Health. It is obligatory to record the number of all individuals that are used in a test, which is inspected every 2 years by Ministerial auditers. Only trained and certified staff are entitled to perform animal tests.

LC/LD50 tests are in principal prohibited, but one can obtain an exemption when you can provide a sound motivation: importance and necessity of the test, no reasonable alternative available, good animal care. We have never experienced restrictions in executing our fish tests, although in principle this regulation can give legal conflicts with the chemical (pesticide/biocide) registration acts, that prescribe an obligatory fish test.

United Kingdom
The regulations here only cover vertebrates and Octopus. There is no prohibition on doing LC50 tests with vertebrates providing one can justify them. For example, one would be crazy to run an expensive chronic fish test if one did not know the concentrations which would produce lethality. We have also used fish lethality tests in a TIE procedure to identify the causes of a massive fish kill which was threatening to wipe out the whole of the River Kennet. It's a balancing of the benefits and disbenefits.

Basically, we are able to justify everything we do in terms of environmental protection, even if it does mean killing fish. The red tape is a bit tedious, but it has not yet stopped us doing what we need to do.

USA

The situation in the US is that we can use animals in research but the process of application and approval is long and difficult and discourages people from doing so. I think that was the intent of the legislation.