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ADVANCES IN THE EVALUATION OF THE TOXICOLOGICAL RISKS OF HERBICIDES TO THE ENVIRONMENT

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Introduction

Assessing potential environmental risks from the use of herbicides is an important part of the process of agricultural practice and the regulatory control of all agrochemicals. This process is necessary to ensure that the herbicides are used in a way that will maximize their usefulness to the user and minimize the risk to the environment. This is to ensure that the environment in the agricultural ecosystem is protected so that it may be used for continued and consistent food and fibre production in the future.

Risk assessment has a crucial role in strategic planning and in helping society to determine environmental priorities. Over the past few years there has been an increased emphasis within several governments and other agencies[1,2,3] to improve the process of ecological risk characterization. In 1992, the Risk Characterization Forum of the U.S. Environmental Protection Agency published a framework for conducting and evaluating ecological risk characterizations[2]. The procedure described in the Framework is essentially similar to that used in other jurisdictions[3] and begins with a problem formulation phase in which the endpoints and the affected environments are defined. This is followed by an analysis phase in which characterizations of effects (dose-response relationships) and exposure are carried out. In the next step of the process, risk characterization, exposure and effects information are characterized and then integrated into a risk estimate. This information, together with scientific uncertainties, and interpretation of the ecological, economic, social and political significance of anticipated effects, is then used by risk managers in making a regulatory decision.

The Agroecosystem and the Environment

When assessing risks from the use of herbicides, or other pesticides, in the environment, it is essential do distinguish between the environment as a whole and that part of the environment that is used for agricultural production - the agroecosystem. When using herbicides, the agroecosystem is clearly defined. In agricultural production, crop management is required in the area planted to the crop or the area used by domestic animals. This agroecosystem is subjected to several levels of management that include the mechanical, cultural and chemical modification. The purpose of crop management is to increase efficiency of production and agriculturalists use all tools at their disposal. Herbicides and other pesticides are only one tool used by agriculturalists and need to be considered in this framework.

An agricultural field is not part of the natural environment, however, it is usually surrounded by natural buffer zones (other agricultural lands, roads, hedges or the required buffers between fields and bodies of water or other areas deemed to require protection). Because the crop area is intensively managed, the ecosystem at risk from herbicides is not the farmers field or the buffer zone around the field, but is the area beyond these boundaries. This important concept needs to be taken into account in the characterization of the impacted ecosystem and particularly in the estimation of exposures to herbicides. The distribution of agricultural land is usually well defined and the nature of the crop (and therefore the pest and the pesticide) is known. Similarly, the type of nontarget ecosystem located close to the agricultural land is also well defined.

While the agroecosystem is highly managed to increase efficiency of production, it is also used by organisms other than humans. These organisms include insects and other invertebrates, mammals, and birds. Some of these may be regarded as pests and therefore subjected to control while others merely use the agroecosystem as habitat or shelter. Obviously, as most agroecosystems are monocultures, and are therefore not suitable habitat for some organisms, the process of agricultural production may exclude certain species from that habitat. However, it is important to remember that this exclusion is a result of the joint effect of all the tools used in agriculture and not just because of the use of herbicides or other pesticides.

Toxicity Assessment of Herbicides

Toxicity is the capability of a substance to produce injury. It is the inherent poisonous potency of a substance and is usually measured under experimental conditions in the laboratory or the field. Toxicity depends on the physical and chemical properties of the substance and the organisms with which it comes into contact. This is where most herbicides have special properties, i.e. they are more toxic to plants than to animals. This selectivity of action is important when assessing risks from the use of these substances.

Toxicity assessment occurs during the early development of a pesticide with toxicological studies on mammals and other organisms. Evaluation of toxicity to mammals is determined by various routes of exposure including, ingestion (oral toxicity), skin or dermal exposure, inhalation, and via the matrix, such as water. This data may take many years to develop and is detailed by other speakers at this meeting[4]. Important information from these tests are data such as LD50 and LC50, however, the NOAEC (No Observed Adverse Effect Concentration, that concentration at which no effects are observed) and the NOAEL (No Observed Adverse Effect Level, that concentration in the diet at which no effects are observed, usually in mammalian studies) are also important

The toxic potential of herbicides and other pesticides to humans is often classified into categories (Table 1) on the basis of toxicity and the dose required to kill a human.

Selectivity of action

Receptor mediated modes of toxicity usually result in high toxicity to organisms that possess the receptor system and lower, background toxicity as a result of their physical or narcotic actions in non-receptor organism. This is the principle behind the selectivity of many pesticides and is clearly seen in the higher sensitivity of algae and plants to herbicides such as atrazine and the other triazines[5, 6] and the obvious selectivity of insecticides affecting the nervous system for animals over plants.

Table 1 - Chemical Hazard Ratings (from the USEPA)

Category	Signal word	Acute toxicity LD50 mg/kg		Lethal oral dose for 70 kg person	Common chemicals and herbicides (active ingredient, not formulation)
		Oral	Dermal		
I: Highly toxic	Danger	0-50	0-200	milligrams to 3.5 g	Sodium hypochlorite (concentrated laundry bleach), paraquat.
II: Moderately toxic	Warning	50-500	200-2,000	3.5 g to 35 g	Naphtha (solvent for paints etc.), 2,4-D.
III: Slightly toxic	Caution	500-5,000	2,000-20,000	35 g to 350 g	Liquid detergent, dicamba, atrazine, hexazinone, trichlorpyr, asulam.
IV: Relatively nontoxic	Caution	> 5,000	> 20,000	>350 g	Baby lotion, fosamine, glyphosate, simazine, picloram, sulfpmeturon, metsulfuron.

Hazards from Herbicides

Hazard is a combination of toxicity and intensity of exposure. Without toxicity or exposure there can be no hazard. A highly toxic substance is without hazard if there is no exposure. A low toxicity substance may be a hazard if intensity of exposure is high. Hazard is usually assessed by comparing exposure to effect concentrations through the use of hazard quotients. These are simple ratios of exposure and effects and may be used to express hazard or relative safety. For example:

$$\textbf{Hazard} = \frac{\textbf{Exposure concentration}}{\textbf{Effect concentration}}$$

The calculation of quotients traditionally has been conducted by utilizing the susceptibility of the most sensitive organism or group of organisms and comparing this to the greatest exposure concentration. This may be made more conservative by the use of a safety (application) factor[7] such as division of the effect concentration by a number such as 20. This is done to allow for unquantified uncertainty in the effect and exposure estimations or measurements.

Hazard assessment

Hazard assessment must take the potential exposure into account. Again, taking the example of humans, it is possible to calculate the amount of various herbicides that would present a hazard to humans. As this involves exposure through various matrices, the consumption of contaminated water, food, etc., must be considered (Table 2). Obviously, these herbicides present a low hazard to humans.

Hazard assessment for herbicides in the environment is conducted in a similar manner. For example, the hazard of a herbicide to fish would be assessed in terms of the toxicity determined from laboratory experiments and the concentration in the environment.

Characterization of exposure

The estimation of pesticide exposure is routinely carried out as part of the hazard assessment process. Although measurements of concentrations of herbicides in the environment are sometimes available, simple models are often used to estimate reasonable high exposure use scenarios. Exposures may result from off-target movement of the herbicide or from direct application to the agricultural field and exposure of organisms that inhabit or utilize the field. Several simple models are available for estimating exposures in the environment.

Table 2 - Hypothetical daily intake by a 70 kg person of matrices containing herbicide residues in relation to the laboratory animal NOAEL divided by a safety factor of 100

Herbicide	Chronic oral NOEL	Matrix consumed to reach NOEL/100			
		Spray mix	Stream water	Foliage area	Animal meat
	mg/kg/d	(ml)[a]	(L)[b]	(m^2)[c]	(kg)[d]
2,4-D	20	0.56	140	0.56	140
Dicamba	5	0.14	35	0.14	35
Fosamine	30	0.84	210	0.84	210
Glyphosate	30	0.84	210	0.84	210
Hexazinone	25	0.7	175	0.7	175
Picloram	20	0.56	140	0.56	140
Triclopyr	30	0.84	210	0.84	210

[a] Assumes the mix contains 2.5 kg/100 L.

[b] Assumes a concentration of 0.1 mg/L.

[c] Assumes 100 L spray mix applied per ha with 10 m^2 foliage per m^2 land area.

[d] Assumes 0.1 mg/kg in meat (most likely to much less than this).

Drift of pesticide sprays is influenced by a number of processes, the most important of which is distance from the site of application. This must be considered in assessing the likely concentration of the agrochemical depositing in an off-target area. There are several approaches to assessing spray drift. One method is to assume that drift is equal to 5% of the application rate (this is the approach used for aerial application in the USEPA GENEC model[8]). Alternatively, actual measurements of drift resulting from the use of pesticides in the field can be used. If measurements are available, they should always be used for assessing hazard as they represent actual concentrations as affected by fate and dissipation in the environment.

Several assumptions can be made with regard to contamination of water with herbicides. The U.S. EPA assumes a water depth of 2 m and a direct overspray with the pesticide[9]. Riley assumes a water depth of 30 cm [10]. In Canada, forest pools are assumed to be 15 cm deep. Rapid degradation (hydrolysis and photolysis) will further reduce the initial concentrations of the pesticide. Drift will also reduce initial concentrations in water, depending on distance.

Assumptions for the calculation of soil concentrations of pesticides are based on a direct application to soil and mixing in the upper 2.5 or 5 cm of soil. If the agrochemical is incorporated in the soil, mixing is assumed to extend to a depth of 20 cm. If a cover crop is present, it may be assumed to intercept 50% of the spray. Rapid degradation (hydrolysis, microbiological breakdown, and photolysis) will reduce these levels further. Drift will also reduce initial concentrations in soil, depending on distance.

Assumptions used in assessing exposure of birds and wildlife are based on estimates used by the FAO[11] of contamination of foliage and seeds and insects. Small birds (up to 100 g) are assumed to eat a maximum of 30% of their body weight in seeds or insects in a day while larger birds (500 g) are assumed to consume 10% of their body weight in a day. Volatilization, photolysis and translocation of the agrochemical in the plant will reduce exposure. Drift will also reduce initial concentrations in foliage and seeds.

The above estimates of exposures are considered to be a reasonable worst case high exposure. In many cases, actual exposures will be much lower, depending on other factors in the environment that reduce concentrations by binding the herbicide to make it less biologically available or reduce concentrations through accelerated degradation.

Risks from Herbicides

Risk is the probability that a substance will cause adverse effects. Risk involves three components, hazard, intensity of exposure, and the probability of exposure. Safety is the inverse of risk but cannot be measured scientifically. Risks from the use of herbicides and other pesticides may result from the direct action of the substance on the non-target organisms or from indirect effects from the alteration of the habitat or food sources for the organism.

Direct effects

Direct effects are easier to interpret as they are more directly related to the data that is available for most herbicides. In this case, toxicity to the non-target species is measured or estimated from closely related organisms and direct responses such as mortality or reduction in yield are derived.

Indirect effects

Non-target toxicity through habitat alteration has been reported in the case of a number of pesticides. Examples of mechanisms through which this may occur are:

The use of herbicides on a large scale may affect animals indirectly as in the case of an alteration in food supply. For example, populations of pocket gophers were reduced drastically following treatment of pasture with 2,4-D. Gophers prefer to eat the seeds of flowering plants and in untreated rangeland will eat a ratio of approximately 82% flowering plants to 18% grasses where the former represent 75% of the plants present. After 2 years of 2,4-D treatment the flowering plants had been reduced to 9% of the plants but the diet of the gophers had only dropped to a 50 - 50 mixture. The gopher population was reduced to 13% of that on untreated range land.

Herbicides are generally non-toxic to insects at the normal dosage rate but some effects have been observed in fields of barley treated with MCPA or MCPB. In most cases the effects of the herbicides is related to their effect on the flora, i.e., an alteration in the ground cover. Predatory Coccinellid larvae (Lady beetle larvae) are sensitive to 2,4-D and use of this herbicide has been correlated with increases in aphid populations which would normally be preyed upon by the larvae.

Bees, although not directly affected may suffer through a lack of sufficient nectar producing plants. Birds generally have a low acute sensitivity to herbicides but may be affected by changes in the flora in the field. Grey partridges in the 2,4-D sprayed barley fields had their numbers reduced because, in the early spring, they are dependent on Collembola as a source of food (representing 40% of the crop contents). The numbers of Collembola were reduced by habitat alteration from 2,4-D spraying. Defoliation and killing of broad-leaf plants in Vietnam reduced the availability of nesting sites and caused a reduction in the numbers of birds in the area. This is probably a long-term effect as the broad-leafed plants have now been replaced by bamboo.

The use of herbicides may have, on occasion, detrimental side effects that are brought about in unusual ways. Inadvertent use of herbicides on the wrong crop and the problems associated with drift of spray cause by far the greatest problem in environmental contamination but can be lessened by careful attention to the technique of spraying as well as changes in the formulation of the herbicides and the addition of stickers, evaporation reducers, etc.

If plants are sprayed with sub-optimal doses of 2,4-D, they are sometimes affected in such a way that high concentrations of nitrate accumulate in the leaves. These crops may then become more attractive to cattle which eat the leaves. In the rumen the nitrate is converted to nitrite.

$$NaNO_3 \xrightarrow{[H]} NaNO_2$$

The nitrite is taken into the blood where it oxidizes the hemoglobin to methemoglobin which does not carry oxygen as easily.

$$\frac{-Fe^{++}}{Red} \quad \frac{NO_2}{\rightarrow} \quad \frac{-Fe^{+++}}{Brown}$$

Should this happen to more than about 75% of the hemoglobin the animal will die as a result of insufficient oxygen reaching the vital organs. The antidote is methylene blue which acts as an intermediate in the reduction of Fe^{+++} by NADPH that is naturally present in the body.

Numerous examples of similar indirect effects on populations and ecosystems exist but not all of these are related to the use of pesticides. Other activities of humans, such as the exploitation of natural resources, agriculture in the general sense and the building of dams, roads, pipelines and cities all cause disruption of the environment and may be responsible for habitat alterations.

Distributional approaches in risk assessment

Hazard assessments use single estimates of environmental concentrations, the quantitative likelihood of occurrence of which is unknown because they have not been placed in a probabilistic framework that is necessary to capture the variable nature of actual environmental concentrations. Recently, procedures have been suggested for estimating exposure that take the natural variation into account as much as possible by providing distributions of environmental concentrations (ECs) rather than single EC values[9]. Such probabilistic EC distributions can be used to estimate how frequently concentrations of a substance will exceed any given concentration threshold for effect or toxicity to organisms in the environment.

In protecting the environment, the range of susceptibility of species to substances also must be taken into account. Traditionally, this has been done by utilizing the susceptibility of the most sensitive organism or group of organisms. In the absence of an adequate range of toxicity tests, these risk assessments may be underprotective, however, where an acceptable range of toxicity data are available, they may be overly conservative and result in unnecessarily restrictive regulations. As with the environmental concentrations, characterization of toxicity should take these ranges of susceptibility into account.

The probability of occurrence of a particular event is, and has been, widely used in the characterization of risk from many physical and medical events in humans (the insurance industry) and for protection against failure in mechanical and civil engineering projects (time between failures, one-in-one-hundred-year floods, etc). This concept is now being applied in ecotoxicological risk assessment for the characterization of both exposures and effects, however, this is being done with some qualifiers. These qualifiers relate to return frequencies of events and the costs of replacement/recovery. Engineers constructing a high-cost bridge would opt for risk protection against rare flooding events such as the 100-year return flood level. This is done to mitigate against frequent costly replacements of the bridge. The same principle has been applied in characterizing high exposure events in ecotoxicological risk assessment but the return frequency protected against is more properly related to the lifecycle and reproductive potential of the affected organisms. Conservative approaches to ecotoxicological risk assessment may use low frequencies of return (for example, one-in-thirty years) to ensure protection of all organisms in situations where knowledge of mode of action or sensitivity of species is limited[9]. However, where more information is available, more realistic return frequencies may be used. The herbicide atrazine is a reversible inhibitor of photosynthesis in plants. Atrazine in surface waters primarily affects phytoplankton and aquatic plants, organisms with short life cycles and high rates of reproduction. Thus, return frequencies of a year or less will allow for recovery, even from rare high-exposure events[5]. Similarly, zooplankton would also be protected by short return frequencies. Protection of longer lived species such as some fish may require consideration of return frequencies of several years but even these may be conservative because of repopulation from unexposed refugia. The example of the more rapid than expected recovery of the biota in the River Rhine from an endosulfan spill is a case in point.

Expressing the results of a refined risk characterization analysis as a distribution of toxicity values rather than a single point estimate is an approach presently being used or recommended by Governments[3, 12] and others[9 13]. A major advantage of this approach is that it uses all relevant single species toxicity data and, when combined with exposure distributions, allows quantitative estimations of risks to organisms. In using overlaps of distributions as assessment endpoints, there is an implied assumption that protecting a certain proportion of species for a certain proportion of occasions will also preserve population and community function. This assumption is consistent with our knowledge of ecotoxicological theory[14 15]. There are some limitations to this approach[9 5]. For example, the choice of protection level (e.g., 90% of species) may not be socially acceptable. Some may view 90% as being overprotective, while others may find that level of risk unacceptable, especially if the 10% of potentially affected species includes organisms of great ecological, commercial, or recreational significance. However, the procedure is such that these species are easily identified from the distribution and this question can be addressed[5].

Because probabilistic risk assessment procedures consider all of the available data rather than a single point estimate or value, they inherently consider uncertainty and avoid the many pitfalls of worst-case scenarios[16].

Ecological Relevance

Although probabilistic risk assessments are data driven and require fewer subjective decisions than deterministic procedures, the criterion for overlap of the effects and exposure distributions requires good judgement. However, unlike most human health risk assessment, ecological risk assessments can be subjected to experimental testing. In many cases, particularly for pesticides, field and/or microcosm/mesocosm studies have been carried out. These studies, can be used to test the decision point of the probabilistic approach. In the case of the atrazine risk assessment[5], over twenty mesocosm and microcosm studies had been conducted and showed that atrazine concentrations of 20 µg/L, or less, resulted in little or no adverse effects on the function of aquatic plant communities, the most sensitive group of organisms to atrazine[5]. Effects, when they were observed, were generally short-lived and did not reduce overall plant biomass and primary productivity. However, as expected, and as discussed above, species composition was affected. Effects, either direct or indirect on organisms other than plants were only seen at concentrations exceeding 50 µg/L. By way of comparison, the 10th centiles of the distributions of the sensitivity data for atrazine were 5.4 µg/L for EC5s in plants, 37 µg/L for the LC50s for all organisms, and 3.7 µg/L for the NOAECs/MATCs[5]. This suggests that use of the 10th centile of the sensitivity distribution as a decision criterion was somewhat conservative.

In addition to testing the hypothesis of the probabilistic assessment criterion, mesocosm studies may be used to test the ecological relevance of the responses to the stressor. As has been pointed out[5], microcosms and mesocosms incorporate the aggregate responses of multiple species and, because species vary widely in their sensitivity to atrazine, the overall response of the community may be quite different from the responses of individual species as measured in laboratory toxicity tests. Studies with microcosms and mesocosms also allow measurement of indirect effects of stressors on other trophic levels. Indirect effects can result from changes in food supply, habitat, or water quality, etc., and, although they can be inferred from laboratory toxicity data, they can be measured directly only in multitrophic systems. The risk assessment of atrazine showed that, although some phytoplankton were the most sensitive organisms to atrazine of the laboratory, there was a considerable range of sensitivities in this taxon[5]. Mesocosm studies showed that resistant taxa tended to replace more sensitive taxa in atrazine-stressed phytoplankton communities, reducing the impact on community productivity and biomass. Similar shifts were also observed in macrophyte communities. As a result, aquatic plant community functions were considerably less sensitive to the effects of atrazine than the most sensitive plant species.

Conclusions

Although the use of probabilistic methods of risk assessment has been advocated in several regulatory jurisdictions[2, 3, 12], this has several implications for regulators and the regulated that may not have been fully appreciated. Probabilistic risk assessment is based on the protection of ecosystem function and the sustainability of community production. Regulators will have to accept that ecosystems have redundancy, resiliency and that functions are conserved even when the ecosystem is under stress. This requires recognition of the differences between human health risk assessment and ecological risk assessment and the acknowledgment of this in regulations and legislative instruments. This may require that regulators become more familiar with some of the basic concepts of ecology and ecotoxicology.

As has been pointed out[17] the use of probabilistic risk assessment does not eliminate judgmental decisions, particularly in relation to the most suitable models to describe data sets with relatively few data points. Because of the need for data, particularly for exposure concentrations, probabilistic risk assessment in ecotoxicology is more easily applied to situations where environmental exposure data are available.

Probabilistic risk assessment offers several regulatory advantages. The process is almost entirely data driven and, the more data available, the less the decision endpoint will change. Thus, regulations will not be governed by the newest low level of effect. The process is data driven, and, given the same data set, the same result will be derived, regardless of the beliefs of the assessor. The system is flexible in that the data may be revisited and reanalyzed to focus risk assessments to certain scenarios with different ranges of exposure or with different species assemblages. By providing distributions of exposure and sensitivity, probabilistic approaches can supply information that is more useful in risk management than deterministic approaches which rely on point estimates or worst-case assessments. The process is also useful in risk management as it allows the rational identification and ranking of higher risk scenarios for risk reduction and site-specific risk mitigation. These advantages do not come without some costs. The probabilistic approach to ecotoxicological risk assessment is data intensive and requires more data than the traditional approach[9]. However, the costs of generating the additional data are, relatively speaking, low, but not insignificant. However, these costs may be compensated for by reduced testing requirements in other areas, such as full life-cycle tests[9]. Because of the relative simplicity of the measurement endpoints required for ecotoxicological risk assessment, costs of testing are generally low.

On balance, probabilistic approaches to ecotoxicological risk assessment offer significant advantages and are likely to be more widely applied in the future. Although these techniques are easy to apply, they require better judgmental skills and, if anything, demand a wider range of expertise than might be expected[5]. They are not the ultimate solution for ecotoxicological risk assessment but they are better than the procedures used in the recent past and represent a significant step forward.

References

1. **NRC**. 1993. Issues in Risk Assessment. National Research Council, National Academy Press, Washington, DC.
2. **Environmental Protection Agency. 1992**. Framework for ecological risk assessment. EPA/630/R-92/001 U.S. EPA, Washington, (1992), 41pp.
3. **Environment Canada.** 1996. *Ecological Risk Assessments of Priority Substances under the Canadian Environmental Protection Act: Resource Document* Draft 1.0 dated March, 1996 and *Ecological Risk Assessments of Priority Substances under the Canadian Environmental Protection Act: Guidance Manual* Draft 2.0 March, 1996. Environment Canada, Ottawa.
4. **Dr. J.A. Guth**. 1997. Evaluation of environmental risks of herbicides: Industry viewpoint. Proceedings of the 21st Brazillian Congress of Weed Science. Caxambu, MG, Brazil, July 6-11, 1997.
5. **Solomon, K.R., D.B. Baker, P. Richards, K.R. Dixon, S.J. Klaine, T.W. La Point, R.J. Kendall, J.M. Giddings, J.P. Giesy, L.W. Hall, Jr., C.P. Weisskopf, and M. Williams.** 1996. Ecological risk assessment of atrazine in North American surface waters. *Environmental Toxicology and Chemistry*, **15**:31-76.
6. **Solomon, K.R. and M.J. Chappel.** 1996. Triazine herbicides: ecological risk assessment in surface waters. In: Triazine Risk Assessment. (Eds. Ballantine, L., McFarland, J., and Hackett, D. ACS Symposium Series. American Chemical Society, Washington D.C. (In press).
7. **CWQG.** 1987. Canadian Water Quality Guidelines and updates. Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers., Ottawa, ON, 1987.
8. **Parker, R.D. and D.D. Rieder**. 1996. The generic expected environmental concentration program, GENEEC. Users manual. Tier one screening model for aquatic pesticide exposure. Environmental Fate and Effects Division, Office of Pesticide Programs U.S. Environmental Protection Agency, Washington DC.
9. **SETAC. 1994**. SETAC Foundation for Education, Report of the Aquatic Risk Assessment and Mitigation Dialogue Group for Pesticides. SETAC, Pensacola, FL (1994).
10. **Riley, D**. 1993. Principles of risk assessment. In:Environmental Criteria for Assessing Agricultural Pesticides. Rijtema, P.E., P.Leeuwangh and M. Liestra (Eds.) Report # 73, The Winand Staring Centre for Integrated Land, Soil and Water Research, Wageningen, Netherlands, 1993.
11. **FAO**. 1989. Revised Guidelines on environmental criteria for the registration of pesticides. Food and Agricultural Organization of the United Nations. Rome, December, 1989 pp.51
12. **Health Council of the Netherlands**. 1993. Ecotoxicological risk assessment and policy-making in the Netherlands - dealing with uncertainties. *Network*, **6(3)/7(1)**:8-11.
13. **Klaine, S.J., G.P. Cobb, R.L. Dickerson, K.R. Dixon, R.J. Kendall, E.E. Smith and K.R. Solomon.** 1996. An ecological risk assessment for the use of the biocide, dibromonitrilopropionamide (DBNPA) in industrial cooling systems. *Environmental Toxicology and Chemistry*, **15**:21-30.

14. **Tillman, D., D. Wedlin and J. Knops.** 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*, **379**:718-720.

15. **Tillman, D.** 1996. Biodiversity: Population versus ecosystem stability. *Ecology* **77**:350-363.

16. **Suter II, G., with L.W. Barnthouse, S.M. Bartell, T. Mill, D. Mackay and S. Patterson.** 1993. Ecological risk assessment. Lewis Publishers, Boca Raton, 1993 pp.538.

17. **Richardson, G.M.** 1996. Deterministic versus probabilistic risk assessment: Strengths and weaknesses in a regulatory context. *Human and Ecological Risk Assessment*, **2**:44-54.