

## 7. THE APPLICATION OF HAZARD ASSESSMENT SCHEMES USING THE WATERTOX TOXICITY TESTING BATTERY

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### 1. Objectives

The development or application of new or existing toxicity ranking systems, based on the use of a battery of tests inspired by the WaterTox Program are presented.

These systems allow for the aquatic toxicity assessment of water-soluble contaminants from different type of matrices.

Application examples are provided for surface waters, groundwater, and pore waters from sediments, as well as complex environmental samples including industrial wastewaters, biosolids from municipal treatment plants, hazardous wastes and waste leachates.

The WaterTox battery, comprising standardized toxicity tests which have undergone intercalibration exercises, is simple to use, scientifically robust, cost-effective and user-friendly. Applications of different Hazard Assessment Schemes using the battery of test approach are discussed.

## 2. Summary

The application of a core battery of WaterTox Program toxicity tests were applied to different types of samples by three South American laboratories. The core battery included the following tests:

- *Lactuca sativa*, 120 h inhibition of germination and root elongation test (Dutka, 1989a);
- *Daphnia magna*, 24-48 h acute lethality test (Dutka, 1989b);
- *Hydra attenuata*, 48-96h acute lethality and sublethality test (Blaise and Kusui, 1997; Trottier et al., 1997); and
- *Pseudokirchneriella subcapitata* -formerly *Selenastrum capricornutum*- 72-h growth inhibition test (Blaise et al., 2000).

Different Hazard Assessment Schemes (HAS) were developed based on existing indexes. In Colombia, the PEEP index (Costan et al., 1993) was used to compare the toxic potential of industrial wastewaters. In Chile, a ranking scheme based on approaches proposed by the National Water Research Institute of Environment Canada (Dutka, 1988; Dutka and Kwan, 1988) and Gent University, Belgium (Persoone et al., 2003) was employed to assess the toxicity of soluble contaminants associated with biosolids from municipal wastewater treatment plants. In Argentina, a classification system for water samples, the Effect-Dilution Average Ratio Index (EDAR), was utilized to assess hazardous waste, leachates, water and sediment pore waters. In this chapter, applications of these Hazard Assessment Schemes are discussed based on the ranking scale of each HAS and toxicity test responses to pure compounds subsequent to an initial round-robin exercise. Some limitations are encountered in applying the test battery especially owing to the lack of sensitivity related to insoluble toxicants. Testing samples concentrated by pre-treatment with solvents proved unhelpful, since high dilutions were then required to avoid carrier toxic effects. Overall, the application of specific HAS schemes with the WaterTox battery of toxicity tests contributed ecotoxicological information that identified the more problematic water samples and wastes in three South American countries. Such information is crucial for subsequent decision-making that will lead to improved protection and conservation of aquatic ecosystems.

### 3. Historical overview and applications

The International Development Research Centre (IDRC, Ottawa, Canada) created an international network of laboratories (WaterTox) whose goal was to identify and use a battery of toxicity tests which were simple and easy to use, affordable, yet sensitive and reliable, for water toxicity testing (Forget et al., 2000). In the original WaterTox battery (Phase I) the following toxicity tests were used:

- onion root bundle growth assay (Fiskesjö, 1993);
- the lettuce seed germination (Dutka, 1989a) 120-h exposure assay (root and seedling length);
- the *Daphnia magna* 48-h lethality test (Dutka, 1989b);
- the *Hydra* 96-h lethality (tulip stage and disintegration of organisms) and sub-lethality (morphological changes: clubbing and shortening of tentacles) assay (Blaise and Kusui, 1997; Trottier et al., 1997);
- the Muta-Chromoplate mutagenicity test (conducted according to instructions provided with this commercial kit); and
- the nematode maturation bioassay (Samoiloff et al., 1980).

Based on criteria evaluating test performance, reproducibility, and user-friendliness, a standardization and calibration exercise was carried out by eight participating laboratories in different countries, involving the testing of 30 blind samples (Phase I). As a result of this exercise, a simplified battery was recommended (Phase II) that called for the use of the lettuce seed germination, *Daphnia* and *Hydra* tests. In addition, an algal test (72-h exposure *S. capricornutum* growth inhibition chronic toxicity), developed within the scope and framework of the WaterTox inter-calibration exercise, was also recommended (Blaise et al., 2000).

Phase II of the exercise involved toxicity screening of environmental (Diaz-Baez et al., 2002) and blind samples (Ronco et al., 2002) with the simplified battery. Critical analysis of each toxicity test was undertaken with the latter samples to evaluate their reliability. This involved looking at such factors as: 1) variability of responses among laboratories to negative controls; 2) conformance with test quality control criteria; 3) false positive responses induced by sample concentration; and 4) variability within and among laboratories of responses to toxic samples. Results indicated that the battery was generally reliable in detecting the presence of toxicity. However, some false positives were identified with a concentrated soft water sample and with the *Lactuca* and *Hydra* (sub-lethal end-point) tests. Probabilities of detecting false positives for individual and combined toxic responses of the four toxicity tests are presented. Overall, inter-laboratory comparisons confirmed good reliability for the battery.

After completion of the WaterTox program, the test battery continued to be applied by laboratories from Argentina, Chile and Colombia to assess different types of environmental matrices. These initiatives facilitated the development or application of new or existing ranking systems that enabled evaluation of the effectiveness of biological treatment for the toxicity reduction of wastes and combined effluents. These studies are described herein.

#### 4. Procedures

##### 4.1 TOXICITY TESTS AND QUALITY CONTROL

Tests employed to describe the studies outlined below on are indicated in Table 1. Each laboratory ran internal quality control charts (U.S. EPA, 1991; Environment Canada, 1999) with known reference toxicants using the following chemicals: Cr(VI) as  $K_2Cr_2O_7$  for *D. magna* and *H. attenuata*; Zn(II) as  $ZnSO_4 \cdot 7H_2O$  for *L. sativa*; and Cu(II) as  $CuSO_4 \cdot 5H_2O$  for *S. capricornutum*. Probit analysis (for *Hydra* and *Daphnia* tests) and non-parametric linear interpolation (for seed and algae tests) were used for the LC/EC/IC50 estimation.

Table 1. Characteristics of small-scale toxicity tests used in the WaterTox battery of tests.

<i>Trophic level</i>	<i>Toxicity test with test species</i>	<i>Assessment and statistical endpoint</i>	<i>Reference</i>
Primary producer	<u>Algal test</u> <i>Selenastrum capricornutum</i>	Chronic sublethal growth inhibition (after a 72-h exposure), IC50	Blaise et al., 2000
Primary producer	<u>Vascular plant</u> <i>Lactuca sativa</i>	Inhibition of germination, root and shoot elongation (after 120-h exposure), IC50	Dutka, 1989a
Primary consumer	<u>Cladoceran test</u> <i>Daphnia magna</i>	Acute lethality (after a 48-h exposure), LC50	Dutka, 1989b
Secondary consumer	<u>Cnidarian test</u> <i>Hydra attenuata</i>	Acute lethality (after a 96-h exposure), LC50 Acute sublethal indicated by morphological changes (after a 96-h exposure), EC50	Blaise and Kusui, 1997; Trottier et al., 1997

##### 4.2 TEST BATTERY APPROACH

The test battery approach used in toxicity testing is now widely advocated internationally for assessing complex mixtures such as municipal and industrial effluents, or hazardous wastes from different sources, as different trophic levels of aquatic biota can be impacted by specific groups of toxicants. However, ranking samples is complex because different tests in the battery will respond to toxicity to varying degrees. One way to resolve this problem is to integrate test responses into a toxicity index that expresses the relative hazard of different samples by a single numerical value.

## 5. HAS case studies

The following sections highlight case studies undertaken independently in three South American countries facing different types of environmental problems related to toxic discharges to aquatic environments.

### 5.1 ARGENTINA

The case study presented here was conducted by the Environmental Research Centre, CIMA, Faculty of Sciences, of the University of La Plata. The more frequently applied tests were those conducted with the *Lactuca sativa* seed germination assay, followed by the *Hydra*, *Daphnia* and *Selenastrum* tests. They were used for the assessment of toxicity from hazardous wastes and waste leachates, sediment pore water and sediment leachates, surface waters and groundwater. An additional test based on  $\beta$ -galactosidase (*in vitro*-free enzyme test) inhibition, known for its sensitivity to metals (Apartin and Ronco, 2001), was also incorporated in the battery.

#### 5.1.1 Effect-Dilution Average Ratio Index (EDAR)

This index (Bulus Rossini et al., 2005), which integrates five tests (four toxicity tests from the WaterTox intercalibration exercise and an enzymatic test sensitive to metals) and six endpoints, was developed as a tool to assess and compare the hazard of water soluble contaminants in surface water bodies and ground water of the coastal region of the Río de la Plata estuary, Argentina (Ronco et al., 1995; 1996; Camilion et al., 2003). The index values were established in such a way that the interval limits for each level were associated with a 20% effect for all tests for a given sample dilution, except for the upper and lower interval of the first and second rank values (Tab. 2). The 0.15 upper interval of the first rank value was based on the consideration that one of the WaterTox toxicity tests produced a negative response and the other four a toxic effect of 20% with the undiluted sample. The responses to three pure compounds from the Phase II WaterTox intercalibration exercise (including the  $\beta$ -galactosidase test) were used to assess the behaviour of the index (Ronco et al., 2002). Application of the index to other types of environmental samples with a reduced battery of three toxicity tests was also conducted using the same principle, but by adapting the toxicity ranking scale.

Different approaches were considered for enhancing the index to rank the ecotoxicity hazard of aqueous samples according to results obtained with the test battery. The selected EDAR index makes use of the sample concentration for each test producing an effect of 20% in line with the following principles:

- The concentration producing a 20% toxic effect (LC/IC/EC20) estimated from the concentration-response curve
- When it is not possible to determine an LC/IC/EC20, the following data can be used:
  - The highest dilution (*i.e.*, lowest concentration) showing a toxic effect of 15% or higher,

Table 2. EDAR index scale for the hazard assessment of aqueous samples using a battery of five toxicity tests.

<b>EDAR Index Interval</b>	<b>Qualitative hazard description</b>	<b>Hazard rank</b>	<b>Response for each lower limit value of the interval</b>
[0-0.15]	Not hazardous	I	Absence of effect with the undiluted sample (100%)
[0.15-0.19]	Possibly hazardous	II	20% effect with the undiluted sample for three tests and no effect in the fourth
[0.19-0.38]	Slightly hazardous	III	20% effect with the undiluted sample with all tests
[0.38-1.9]		IV-1	20% effect in the 50% dilution with all tests
[1.9-3.8]	Hazardous	IV-2	20% effect in the 10% dilution with all tests
[3.8-19]		IV-3	20% effect in the 5% dilution with all tests
[19-38]		V-1	20% effect in the 1% dilution with all tests
[38-189]	Very hazardous	V-2	20% effect in the 0.5% dilution with all tests
> 189	Extremely hazardous	VI	20% effect in the 0.1% dilution with all tests

- The undiluted sample (100%), when toxic responses at this concentration are below 15%; and
- Dilutions producing a 100% toxic effect are not used in the index calculation.

To calculate an EDAR value for the given battery, each estimated effect is divided by the corresponding dilution. Since there is no evidence to support a difference in importance between each toxicity test, the same weight was assigned to all the tests, except for the  $\beta$ -galactosidase assay. The highest sample concentration compatible with this test is a 50% v/v dilution, and it was assigned a weight of 0.5. For the tests with more than one end point, the weight is equally divided between all the end points assessed (*i.e.*, for *Hydra*, with two end points, the total weight is 1 and each end point has a weight of 0.5). Since a 0% effect value could occur at a high dilution (a very intense toxic effect), the value of 1 unit has been added to the measured effect before the quotient is calculated in the formula below.

$$EDAR = \frac{\sum_{i=1}^n p_i \cdot ((e_i + 1) / d_i)}{\sum_{i=1}^n p_i} \quad (1)$$

Where:

- $p_i$  is the weight assigned to the endpoint assessed,
- $e_i$  is the measured effect corresponding to a  $d_i$  dilution,
- $n$  is the number of tests/end points in the battery.

A ranking scale of nine levels ranging from 'non-hazardous' to 'extremely hazardous' was developed (Tab. 2). The rationale behind the EDAR index is based on averaging out the ecotoxic effects of a given aqueous sample.

Since toxicity assessment of environmental samples do not always yield data conducive for the plotting of a concentration-response curve, quantitative response measurements are sometimes impossible to calculate. To compensate for these shortcomings, the EDAR index averages the % effect with the dilution producing this measured effect, hence normalizing the data from the different tests. Whenever sufficient data were available to obtain a concentration-response plot, we selected the sample concentration used in the index calculation as the one producing a 20% effect on the exposed test organisms. This 20% effect generally corresponds to the lowest concentration indicative of significant differences between negative controls and sample effects, based on the results produced with the WaterTox intercalibration exercise (Ronco et al., 2002).

The ranking scale limit values of the index were set considering the results that would be obtained if all tests yielded a response of 20% to the same concentration or dilution. Each interval of the reference scale was arbitrarily fixed according to valued judgment taking into account the authors' experience.

### 5.1.2 Application of the EDAR Index to a case study of surface water and groundwater pollution

Samples investigated with the five toxicity tests from the battery were surface water (*i.e.*, El Gato -S1 and 2-, Martin -S6- and Carnaval -S10- streams, Oeste Canal -S3-, water intake for the treatment plant -S7- and near the sewers discharge -S4- both from the Río de la Plata) and groundwater (S8 and 9), all the sites corresponding to the south eastern sector of coastal area of the Río de la Plata (Ronco et al., 1996, 2001; Camilion et al., 2003), and tap water (S5) with conventional treatment (see the location of sampling points in relation to possible contaminant sources in Figure 1). Physico-chemical parameters from all samples were within the following ranges: conductivity 0.3-1.8 mS/cm; hardness 50-450 mg CaCO<sub>3</sub>/L; dissolved oxygen from non detectable to 8.8 mg/L; DOC mg/L < 20-82 mg/L; alkalinity 100-470 mg CaCO<sub>3</sub>/L (low dissolved oxygen concentrations and higher DOC and conductivity was detected in surface waters close to contamination sources). Blind positive (*i.e.*, Hg(II) and 4-Nitroquinoline-*N*-oxide) and negative (soft water) samples from the Phase II WaterTox intercalibration exercise (Ronco et al., 2002)

and methanol 2% were also tested and ranked using the EDAR index. Scoring results are summarized in Table 3. Samples from surface water bodies considered hazardous according to the scoring system correspond to sectors associated with direct industrial or urban contaminant discharges. Also, potential health hazard from groundwater samples was found to be related to chemical contamination from intensive agriculture. Tap water was sampled from an old lead water pipe. The pure compound index values were clearly higher and in a class apart from the rest of the samples. As expected, no positive toxic responses to the blind negative samples (results not shown in Table 3) were observed in any of the tests in the test battery.

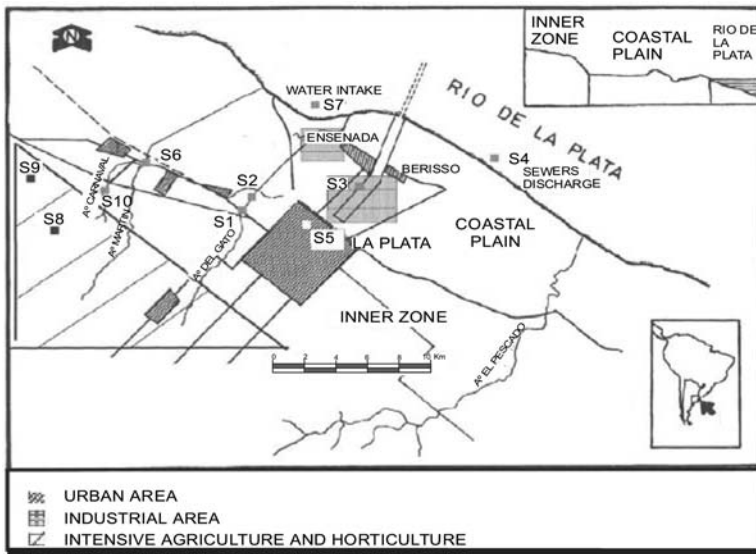


Figure 1. Study area with indication of surface water and groundwater sampling points and type of activity in each sector.

### 5.1.3 Ranking the toxicity of soluble toxicants in several types of matrix leachates and wastes with a reduced battery of tests.

Owing to possible future restrictions that could preclude the testing of samples with all five toxicity tests, we evaluated the index response with a reduced battery of toxicity tests. The basic rule for the selection of tests in a reduced battery was to maintain one primary producer, one primary consumer and a secondary consumer. The test combinations of two selected reduced batteries were: *Hydra*, *Daphnia* and *Lactuca* tests (H-D-L) and *Hydra*, *Daphnia* and *S. capricornutum* (H-D-S) tests



(Tab. 4). These batteries were applied for ranking hazardous waste, pore water and leachates from sediments and wastes. The scale was prepared using the same criteria as previously described (see Section 5.1.1).

Table 3. EDAR index application for the hazard assessment of water samples and pure compounds using a battery of five toxicity tests. Effect:dilution ratio values are indicated for each test.

Sample identification	Algal assay <sup>a</sup>	H-L <sup>b</sup>	H-SL <sup>c</sup>	DM <sup>d</sup>	LS <sup>e</sup>	E <sup>f</sup>	EDAR Index	Sample Hazard Rank <sup>g</sup>
Environmental samples*								
S1	0.36	0.65	1.0	0.59	0.88	0.01	0.59	IV-1
S2	0.41	0.56	2.9	0.67	0.52	0.01	0.84	IV-1
S3	0.42	0.54	10.10	0.04	0.14	0.28	1.92	IV-2
S4	0.01	0.31	1.3	0.82	0.48	0.01	0.48	IV-1
S5	0.68	0.01	0.01	0.44	0.26	0.35	0.29	III
S6	0.01	0.01	0.17	0.70	0.73	0.01	0.27	III
S7	0.01	0.01	0.28	0.01	0.16	0.01	0.08	I
S8	2.2	0.01	0.06	0.01	1.0	0.01	0.70	IV-1
S9	0.32	1.5	1.5	0.01	0.80	0.01	0.54	IV-1
S10	0.01	0.01	0.01	0.01	0.30	0.01	0.06	I
Pure compounds								
Hg(II) 5 mg/L	90	78	631	228	4	155	198	VI
4-NQO 2 mg/L	300	1.2	8.4	0.43	0.42	0.28	52	V-2
Methanol 2%	2.5	0.01	-	0.01	0.32	0.99	0.76	IV-1

\* Sites correspond to the south eastern sector of coastal area of the Río de la Plata (Ronco *et al.*, 1996, 2001, Camilion *et al.*, 2003): El Gato -S1 and 2-, Martín -S6- and Carnaval -S10- streams, Oeste Canal -S3-, water intake for the treatment plant -S7- and near the sewers discharge -S4- both from the Río de la Plata), and groundwater (S8 and 9), see Figure 1.

a) *Selenastrum capricornutum* assay (Blaise *et al.*, 2000).

b) *Hydra attenuata* lethality assay (Blaise and Kusui, 1997; Trottier *et al.*, 1997).

c) *Hydra attenuata* sublethal assay (Blaise and Kusui, 1997; Trottier *et al.*, 1997).

d) *Daphnia magna* assay (Dutka, 1989b).

e) *Lactuca sativa* assay (Dutka, 1989a).

f) Enzyme assay with  $\beta$ -galactosidase (Apartin and Ronco, 2001).

g) See Table 2 for details.

Results of the EDAR index application to these types of matrices are provided in Table 5. Samples were selected for toxicity screening to ensure the presence of different types of contaminants commonly present in complex wastes (*e.g.*, hydrocarbons, DOC, nutrients, ammonia, inorganic anions and cations, pesticides) and other matrices (*e.g.*, sediments and sludges, solid materials, liquid

phases). Water samples and pure compounds studied with the complete battery (Tab. 3) were also scored with the reduced batteries (H-D-L and H-D-S) for comparison (Tab. 5).

The comparison of the EDAR index values obtained for the same samples with the complete and reduced batteries (H-D-L and H-D-S) indicated a good agreement thus supporting the use of a reduced battery of tests when necessary.

Table 4. EDAR index scale for hazard assessment of aqueous samples using two reduced test batteries, H-D-L<sup>a</sup> and H-D-S<sup>b</sup>, each with three toxicity tests.

<b>EDAR Index Interval</b>	<b>Qualitative Hazard description</b>	<b>Hazard rank</b>	<b>Response for each lower limit value of the interval</b>
[0-0.14]	Not hazardous	I	Absence of effect with the undiluted sample (100%)
[0.14-0.21]	Possibly hazardous	II	20% effect with the undiluted sample for two tests and no effect in the third
[0.21-0.42]	Slightly hazardous	III	20% effect with the undiluted sample with all tests
[0.42-2.1]	Hazardous	IV-1	20% effect in the 50% dilution with all tests
[2.1-4.2]		IV-2	20% effect in the 10% dilution with all tests
[4.2-21]		IV-3	20% effect in the 5% dilution with all tests
[21-42]	Very hazardous	V-1	20% effect in the 1% dilution with all tests
[42-210]		V-2	20% effect in the 0.5% dilution with all tests
> 210	Extremely hazardous	VI	20% effect in the 0.1% dilution with all tests

<sup>a</sup> *Hydra*, *Daphnia* and *Lactuca* tests.

<sup>b</sup> *Hydra*, *Daphnia* and *S. capricornutum* tests.

It was observed that EDAR index values and intervals (Tables 2 and 4) did not change markedly with the deletion of two toxicity tests. Results of applying the EDAR index to waste samples indicate that values and ranks relate to the solubility of toxicants in aqueous phases. Based on this evaluation, wastes from photographic and X-Ray laboratories were observed to be extremely hazardous in contrast to hydrocarbon-containing waste leachates, described as either slightly hazardous or hazardous. The existence of sub-levels for an equivalent hazard description allows for better sample discrimination (*e.g.*, Pharmaceutical solid waste leachate *versus* liquid waste with pesticides in Table 5).

Table 5. Results of applying the EDAR index for hazard assessment of waste samples, sediment extracts or pore water and pure compounds using the reduced battery of tests.

<i>Sample identification</i>	<i>EDAR Index</i>	<i>Sample rank</i>	<i>Hazard description</i>	<i>Battery used</i>
<b>Industrial waste samples</b>				
Soil with hydrocarbons from land farming (leachate)	1.23	IV-1	Hazardous	H-D-L
Sludge with oil, grease and hydrocarbons (leachate)	0.33	III	Slightly hazardous	H-D-L
Food industry sludge (pore water)	0.36	III	Slightly hazardous	H-D-L
Food industry solid waste (leachate)	0.29	III	Slightly hazardous	H-D-L
Food industry solid waste (pore water)	0.72	IV-1	Hazardous	H-D-L
Food industry liquid waste	0.02	I	Not hazardous	H-D-L
Photoshop liquid waste	508	VI	Extremely hazardous	H-D-L
Photoshop liquid waste II	117	V-2	Very hazardous	H-D-L
Pharmaceutical solid waste (leachate)	0.70	IV-1	Hazardous	H-D-L
Liquid waste with pesticides	10.2	IV-3	Hazardous	H-D-L
<b>Pure compounds</b>				
Hg(II) 5 mg/L	235	VI	Extremely hazardous	H-D-L
Hg(II) 5 mg/L	257	VI	Extremely hazardous	H-D-S
4-NQO 2 mg/L	2.7	IV-2	Hazardous	H-D-L
4-NQO 2 mg/L	33	V-1	Very hazardous	H-D-S
4-NQO 2 mg/L	2.6	IV-2	Hazardous	H-D-L
4-NQO 2 mg/L	78	V-2	Very hazardous	H-D-S
Methanol 2%	0.11	I	Not hazardous	H-D-L
Methanol 2%	0.83	IV-1	Hazardous	H-D-S
<b>Environmental samples*</b>				
S1	0.79	IV-1	Hazardous	H-D-L
S1	0.66	IV-1	Hazardous	H-D-S
S2	1.2	IV-1	Hazardous	H-D-L
S2	1.1	IV-1	Hazardous	H-D-S
S3	2.7	IV-2	Hazardous	H-D-L
S3	2.8	IV-2	Hazardous	H-D-S
S4	0.72	IV-1	Hazardous	H-D-L
S4	0.60	IV-1	Hazardous	H-D-S
S5	0.18	II	Possibly hazardous	H-D-L
S5	0.28	III	Slightly hazardous	H-D-S
S6	0.4	III	Slightly hazardous	H-D-L
S6	0.22	III	Slightly hazardous	H-D-S
S7	0.11	I	Not hazardous	H-D-L
S7	0.076	I	Not hazardous	H-D-S
S8	0.28	III	Slightly hazardous	H-D-L
S8	0.56	IV-1	Hazardous	H-D-S
S9	0.97	IV-1	Hazardous	H-D-L
S9	0.85	IV-1	Hazardous	H-D-S
S10	0.08	I	Not hazardous	H-D-L
S10	0.01	I	Not hazardous	H-D-S

\* Sites correspond to the south eastern sector of coastal area of the Río de la Plata (see Fig. 1).

When comparing the scores of the water samples and reference toxicants in both scales, the EDAR index description was similar when using the complete battery (Tab. 3) and the reduced H-D-S battery (Tab. 5). Some differences were observed, however, and these were mainly attributable to the pure organic compounds, when the complete and H-D-L reduced batteries are compared. These differences could be associated with a lower sensitivity of the seed test to toxicants and the weight assigned to this assay in the EDAR equation. One notable example is that for methanol (full battery EDAR index = 0.76, Table 3; reduced battery H-D-S EDAR index = 0.83, Table 5; reduced battery H-D-L EDAR index = 0.11, Table 5). Reduction of the number of toxicity tests within a battery certainly favours cost-effectiveness, but selection of those maintained in a reduced battery should be given careful consideration in order to avoid lowering the EDAR index toxicity detection potential. Future applications with different classes of chemical compounds will further explore those factors capable of influencing EDAR index values (*i.e.*, use of full and reduced batteries and toxicity test weight factors) in order to optimize this simple and user-friendly toxicity scale.

## 5.2 CHILE

One goal of the Chilean Government is the treatment of all domestic wastewaters by the year 2010. The generation of 220 tons year<sup>-1</sup> of sludge is expected as a treatment by-product (SISS, 2003). At the University of Chile a team of investigators from different centres is carrying out studies on land application of sewage sludge and biosolids, considering their sanitary quality, heavy metal content and bioavailability, as well as their ecotoxicity. Their main objectives are to generate information for the environmental administration officials that are setting specific regulations for agricultural use.

### 5.2.1 HAS description

To assess soluble contaminants associated with sewage sludge and biosolids from different municipal treatment plants in Chile, a core battery of toxicity tests including *D. magna*, *H. attenuata* and *L. sativa* was used. Two Hazard Assessment Schemes (HAS) toxicity ranking systems were applied to categorize sample toxicity. The first scheme [HAS1] is based on a point ranking system that integrates toxicity data obtained for different tests (Dutka, 1988; Dutka and Kwan, 1988; Dutka, 1993; Castillo et al., 2000). This ranking depends on the number of tests and the weight assigned to each one. The scale comprises five degrees of hazard and ranges from “non toxic”, to “extremely toxic”. The range scheme used in this study was adapted to the three toxicity tests applied here (Tab. 6). Because of the generally lower sensitivity responses elicited with the *L. sativa* toxicity test in response to chemical contaminants, a higher score was allocated to it as compared to the *D. magna* and *H. attenuata* tests. Essentially, higher scores corresponded to more toxic samples with this ranking system.

Table 6. Point allocation scheme for sample ranking and hazard classification based on a toxicity test core battery [HAS 1].

Ranking interval		Test score			Total battery score	Hazard description
L(I)C50%	TU*	<i>D. magna</i>	<i>H. attenuata</i>	<i>L. sativa</i>		
> 90	< 1.1	0	0	0	0	Non toxic
90 – 75	1.1–1.33	1	1	1 – 5	3	Slightly toxic
74.9 – 50	1.34 – 2	2	2	5	6 – 9	Toxic
49.9 – 25	2.01 – 4	4	4	9	10 – 17	Highly toxic
< 25	> 4	6	6	13	18–25	Extremely toxic

\*TU (Toxic Units) =  $[1/(L(I)C50)] \times 100$ .

Table 7. Hazard classification scheme for wastes discharged into the environment [HAS 2].

Class	Hazard description	Characteristics
I	No toxicity	- none of the tests show a toxic effect (< 0.4 TU)
II	Slight toxicity	- LOEC is reached at least for one test - the effect level is below 50% (0.4 - < 1TU)
III	Toxicity	- the L(I)C50 is reached in at least one test - in the 10-fold dilution of sample, the effect is lower than 50% (1-10 TU)
IV	High toxicity	- the L(I)C50 is reached in the 10-fold dilution for at least one test - in the 100-fold dilution of sample, the effect is lower than 50% (> 10-100 TU)
V	Very high toxicity	- the L(I)C50 is reached in the 100-fold dilution for at least one test (> 100 TU)

\* TU (Toxic Units) =  $[1/(L(I)C50)] \times 100$ .

The second scheme [HAS2], proposed by Persoone et al. (2003), is based on toxicity responses of one or more tests applied to wastes, and involves two steps: (i) an acute ranking in five classes (Tab. 7) and, (ii) a weight score for each toxicity class. The class describes hazard from “no toxicity”, if no toxic effects are detected in a sample, to “very high toxicity” when toxic effects for a 100-fold dilution of sample are observed. The class weight quantifies the degree of toxicity in that class. The weight score is expressed in percentage (%), and ranges from 25% - if only one test of the battery reaches the toxicity level of the class - to 93% - if all tests but one reach it. For calculating the class weight, an allocation of a test score is applied for each toxicity test of the battery (Tab. 8). Then, the total score is divided by the total

number of tests. This result is then divided by the maximum particular score obtained, and expressed as a percentage. The higher the weight score obtained, the more toxic hazard the class represents (Persoone et al., 2003). For example, sample AS1-b (HAS2 classification results given in Table 10) yielded the following classification based on its bioanalytical data:

- Toxic units of 28.3 (*D. magna*), 556 (*H. attenuata*) and 30.6 (*L. sativa*) giving individual scores of 3, 4 and 3, respectively (see Tab. 8), thereby placing this sample in class V (on the basis of class criteria outlined in Table 7).
- Sample score =  $[3 + 4 + 3] \div 3$  bioassays = 3.33.
- Class weight % =  $[3.33 \times 100] \div 4$  (the highest score reached by the *H. attenuata* result of 556 TU, as per Table 8 criteria) = 83.3.

*Table 8. Score allocation based on the toxic effect of each core battery bioassay for class weight calculation [HAS 2].*

<i>Toxic effect</i>	<i>Score</i>
No significant toxic effect (< LOEC)	0
LOEC < % effect < L(I)C50 (= < 1 TU)	1
1 – 10 TU	2
10 – 100 TU	3
> 100 TU	4

### *5.2.2 Application of the HAS schemes to biosolids toxicity*

This study included sludge samples from five different wastewater treatment facilities: (i) one stabilization pond (SP), (ii) two conventional activated sludges (AS), (iii) one compact activated sludge (CAS), and (iv) one trickling filter (TF). The conventional AS plants treat sewage produced by close to two and a half million people; the sludge obtained is anaerobically digested, mechanically dewatered, and dehydrated in sand drying beds. The other plants are located in small towns (~ 25,000 inhabitants). Sludge from the SP is auto-digested in the bottom of the pond, remaining there for approximately one year prior to being extracted and air dried; the TF sludge is anaerobically digested in tanks, and dried in conventional sand drying beds; the CAS sludge is not treated.

A total of eight sludge samples and two soils to be amended with sludge were tested with the core testing battery. In addition, two amended soils with AS1 sludge applied in rates 0, and 30 tons per hectare ( $\text{ton ha}^{-1}$ ), incubated during 60 days for agricultural use, were also analyzed. Sludges and soils were air-dried and sieved through a 2 mm mesh-size polyethylene sieve. Portions of the < 2 mm fractions from sludges and soils were ground in an agate mortar and stored in polyethylene sealing bags. Forty g of sludges, soils and amended soils were extracted with the respective culture media from each toxicity test, using a ratio of 1:4. The mixture was shaken at 180 rpm for one hour and centrifuged under refrigeration at 3000 rpm for 20 min. The supernatant was then kept for toxicity testing.

Results of global acute toxicity of sludge and soils (dry-weight basis) are presented in Table 9. According to HAS1, independent of source and moisture, all sludges were classified as “extremely toxic”, reaching the maximum battery score (25 points). Neither of the soils exerted toxic effects on the bioassay battery (0 points). In contrast, “high toxicity” was found in soils (12 points), after two months of application of the final sludge (biosolid) from one of the activated sludge treatment plants, at the rate 30 tons ha<sup>-1</sup> (dry-weight basis).

Table 9. Sewage sludge and agricultural soil toxicity<sup>1</sup> [HAS 1].

Sample	Moisture %	<i>D. magna</i> LC50-48h		<i>H. attenuata</i> LC50-96h		<i>L. sativa</i> IC50-5d		Total score	Hazard description
		(%)	UT	(%)	UT	(%)	UT		
SP	28.3	9.46	10.5	2.48	40.3	2.6	38.5	25	Extremely toxic
CAS	5.9	9.3	11.6	0.11	909	7.37	13.6	25	Extremely toxic
AS <sub>1a</sub>	7.0	3.5	28.3	0.32	316	3.6	27.5	25	Extremely toxic
AS <sub>1b</sub>	8.4	3.5	28.3	0.18	556	3.3	30.6	25	Extremely toxic
AS <sub>1c</sub>	78.0	1.84	54.5	0.15	667	1.02	98.0	25	Extremely toxic
AS <sub>1d</sub>	65.4	1.48	67.6	0.14	714	1.17	85.5	25	Extremely toxic
AS <sub>2a</sub>	34.7	1.26	79.4	0.1	1000	2.17	46.1	25	Extremely toxic
TF	98	3.8	26.3	0.13	769	5.1	19.6	25	Extremely toxic
Soil <sub>1</sub>	2.3	>100	<1.1	>100	<1.1	>100	<1.1	0	Non toxic
Soil <sub>2</sub>	1.3	>100	<1.1	>100	<1.1	>100	<1.1	0	Non toxic
Soil <sub>1</sub> <sup>2</sup>	2.5	17.4	5.8	16.5	6.1	>100	<1.1	12	Highly toxic
Soil <sub>2</sub> <sup>2</sup>	1.2	17.3	5.8	15.1	6.6	>100	<1.1	12	Highly toxic

<sup>1</sup>Dry-weight basis.

<sup>2</sup>Amended soil with final sludge (AS<sub>1a</sub>) of conventional activated treatment sludge (rate 30 tons ha<sup>-1</sup> x 60 days).

Although HAS 1 scheme cannot discriminate into different sub-categories the tested sludge samples (and therefore their relative toxicity), the results are of interest to set acceptable toxicity levels in specific regulations for sludge land application and agriculture reuse. The tested sludge comes from different types of environments (*i.e.*, small towns with mining and agriculture as their main productive activities, and a large city with a great diversity of economical activities), and also different types of sewage treatment, showing similarly high toxicity profiles, posing a potential risk of contamination to surface water and groundwater.

Similarly, the HAS2 classification system confirmed the high toxicity of sludges and the negative responses of both soils (Tab. 10). Most sludges fell into class V, with a weight of 83.3 %. SP sludge proved to be somewhat less toxic with a weight of 100% into class IV. In contrast, the hazard toxicity of amended soils decreased by two levels, falling into class III, with a weight of 66.7%.

Table 10. Toxicity of sewage sludge and amended soils using [HAS 2].

<i>Sample</i>	<i>Class</i>	<i>Hazard description</i>	<i>Class weight (%)</i>
SP	IV	High toxicity	100
CAS	V	Very high toxicity	83.3
AS1-a	V	Very high toxicity	83.3
AS1-b	V	Very high toxicity	83.3
AS1-c	V	Very high toxicity	83.3
AS1-d	V	Very high toxicity	83.3
AS2-a	V	Very high toxicity	83.3
TF	V	Very high toxicity	83.3
Soil <sub>1</sub>	I	No toxicity	--
Soil <sub>2</sub>	I	No toxicity	--
Soil <sub>1</sub> +AS1-a <sup>2</sup>	III	Toxicity	66.7
Soil <sub>2</sub> +AS1-a <sup>2</sup>	III	Toxicity	66.7

<sup>1</sup>Dry-weight basis.

<sup>2</sup>Amended soil with digested sludge of conventional activated sludge treatment (rate 30 tons ha<sup>-1</sup> x 60 days).

Using the HAS1 framework, the *H. attenuata* test yielded the most sensitive toxic responses for all types of sludges. However, although the sensitivity of *D. magna* and *L. sativa* was of the same order of magnitude, classification of sludges as being “extremely toxic” (HAS1, Tab. 9) was in part attributable to the latter test, because of its high test score attribution (Tab. 6). In amended soils, both *D. magna* and *H. attenuata* assays generated maximum test scores (Tab. 6). In this instance, the negative response of *L. sativa* tended to reduce the hazard level of this matrix. The HAS2 classification scheme was similar in its ratings of samples and no major differences with respect to HAS1 were observed (Tab. 10).

In general, both hazard schemes were found to be simple and easy to apply and they can be considered complementary. When toxicity is present, both can discriminate between high, medium, low and absence of hazardous effects on tested organisms. HAS1 takes into account the response of each toxicity test included in the battery, assigning a particular score related to their respective response to toxicants. In contrast, HAS2 classifies hazard level based on the response of each test, but also includes a weight factor within a toxic class. Again, HAS1 attributes a toxic hazard based on all test scores while HAS2 gauges the hazard level. Based on the HAS1 scheme, all sewage sludge samples reached the highest classification, because their score was > 4 TU in all tests (Tab. 6). Because of the class and weight criteria imposed by the HAS2 scheme (Tab. 7), it appears to offer better possibilities to discriminate sludges on the basis of their toxic properties (Tab. 10).

Future studies should strive to improve upon these HAS schemes so as to better discriminate between highly toxic samples by separating them into sub-classes. This, in turn, will allow for the development of more precise criteria for the disposal of



such hazardous wastes. Presently, some of the samples investigated, whose toxicity demonstrates effects at 1:10 and 1:1000 dilutions, are all grouped in the same class rank as “highly toxic”. There is room for improvement in future optimization of HAS schemes to refine their judgement in terms of toxicity classification.

### 5.3 COLOMBIA

#### 5.3.1 Principle of HAS and toxicity tests employed

The proposed hazard assessment scheme (HAS) used in Colombia is a ranking system where toxicity data obtained from the application of a test battery enables one to determine the degree of toxicity of liquid samples on a relative basis. Test battery results are then integrated into the Potential Ecotoxic Effects Probe (PEEP) index formula developed by Environment Canada for the comparison of wastewaters (Costan et al., 1993). This index can be applied to evaluate the potential toxicity of industrial and municipal wastewaters, and to assess the effectiveness of toxicity abatement measures for effluents. This procedure is easy to apply and can be used with different batteries of tests (see Chapter 1 of this volume).

As its principle, the PEEP index integrates the responses of a test battery of toxicity tests and determines the relative toxic loading contribution of a series of effluents to the toxic loading of the same receiving environment on a comparative basis using organisms from different trophic levels and taxonomic groups. In Colombia, the previously described toxicity tests were complemented with the agar plate method for rapid toxicity assessment of water-soluble and water-insoluble chemicals (Liu et al., 1991). In the agar plate method, pre-dried agar plates are thinly coated with a quantitative amount of fresh *Bacillus cereus* culture and the seeded plates are spotted with test chemicals at known concentrations. The plates are incubated at the optimal growth temperature for four hours and the diameter of the inhibition zone can be measured.

#### 5.3.2 Determination of Effluent Hazard Potential

Hazard potential for each effluent was calculated using a mathematical formula (the PEEP index) proposed by Costan et al. (1993). This formula integrates the ecotoxic responses of the battery of tests before and after a biodegradation step. Toxicity test endpoint responses are first transformed to toxic units. The product of effluent toxicity and effluent flow ( $\text{m}^3/\text{h}$ ) gives the toxic loading value. The log 10 value of an effluent's toxic loading corresponds to its PEEP index. In order to rank the effluents a toxicity classification scale is generated (Tab. 11).

$$P = \log_{10} \left[ 1 + n \left( \frac{\sum_{i=1}^N T_i}{N} \right) Q \right] \quad (2)$$

Where:

P = PEEP value,

n = number of endpoints exhibiting toxic responses,

N = maximum number of obtainable toxic endpoints,

$T_i$  = Toxic Units from each test, before and after biodegradation,  
 $Q$  = effluent flow in  $m^3/h$ .

Table 11. PEEP index scale for the hazard assessment of wastewater samples using a reduced test battery.

<i>PEEP index values</i>	<i>Toxic classification</i>
< 1.99	Practically non-toxic
2- 2.99	Slightly toxic
3-3.99	Moderately toxic
4- 4.99	Highly toxic
> 5	Very highly toxic

### 5.3.3 Application of the PEEP Index to a case study of industrial wastewaters

The Bogotá River basin is 375 km long and drains an area of about 6107 km<sup>2</sup>. The river receives wastewater from a wide variety of industries, such as tanneries, organic and inorganic chemical production, metal plating, textile production, mining, agrochemical production, as well as sewage from the City of Bogotá and many other smaller municipalities. In 1995, the Colombian Ministry of the Environment, through the Regional Corporation for the control of the river, undertook a program to improve the water quality in the Bogotá River basin. The goal of this program was to reduce by 80% the load of organic compounds and toxicant concentrations by 50% that were being discharged into the Bogotá river.

To reduce the inflow of toxic substances into basin waters efficiently, the Corporation required information on which effluents posed the greatest hazard to the river. While chemical data on many of the effluents were available, their diverse composition was difficult to interpret in terms of hazard potential. Moreover, hazard is not only linked to chemical composition of an effluent, but also on the toxic effects it can have on a variety of freshwater organisms. Adverse effects on biota are also influenced by the volume of wastewater discharged at different times of the year. Hence, the Corporation searched for a cost-effective approach, based on ecotoxicological principles, to rank the various effluents (there are several hundred sources), in terms of their toxic loading, so that subsequent efforts (such as clean up actions) could be prioritized.

In light of these concerns, the National University of Colombia initiated a pilot study, through the application of the PEEP toxicity index to the Bogotá River. This study began by collecting wastewater samples from three effluent sources typical of industries with highest toxic load to the river; tanneries, a thermal power plant and a chlorine production. The assessment was conducted using a battery of three toxicity tests (*i.e.*, the *D. magna* 48-h motility inhibition assay, the Agar plate bacterial growth inhibition test and the *S. capricornutum* 72-h growth inhibition test); the results are presented below in Table 12. The PEEP index clearly identified the chlorine plant effluent as the most toxic for the receiving environment as it

contributes, on its own, close to 98% of the toxic loading generated by this series of five effluents.

Another important industrial sector investigated was the textile industry. Ten different effluent samples were collected and each wastewater was characterized by standard chemical analyses as well as by the toxicity test battery. In this case, the test species included *Daphnia*, *Hydra* and *Lactuca*. Toxicity endpoint values were first transformed into toxic units (TU), a quantitative expression reflecting the resulting toxic potential of all chemical contaminants present in an effluent sample. Subsequently, their PEEP values were determined (Tab. 13).

Table 12. PEEP index characteristics for five effluent samples, and percentage contribution (%) of each effluent to total toxic charge.

<b>Industrial effluent</b>	<b>Effluent flow (m3/h)</b>	<b>Toxic print<sup>1</sup></b>	<b>Toxic charge<sup>2</sup></b>	<b>%</b>	<b>PEEP value</b>
Tannery	0.001	352	0.3	0.002	0.11
Tannery	0.001	443	0.4	0.003	0.14
Thermal Power Plant	25.2	1.2	29	0.23	1.48
Thermal Power Plant	248.4	1.1	279	2.17	2.45
Chlorine Plant	0.429	29172	12520	97.6	4.10
Total toxic charge			12829		

<sup>1</sup>  $n (\sum T_i / N)$  in the PEEP formula  
<sup>2</sup> Effluent flow x Toxic Print in the PEEP formula

Table 13. PEEP index characteristics for ten textile effluent samples, and percent contribution (%) of each effluent to the total toxic charge.

<b>Plant</b>	<b>Effluent flow (m<sup>3</sup>/h)</b>	<b>Toxic print<sup>1</sup></b>	<b>Toxic load<sup>2</sup></b>	<b>%</b>	<b>PEEP value</b>
1	0.67	29.2	19.5	0.2	1.31
2	0.83	33.9	28.2	0.2	1.47
3	5.2	11.6	61.0	0.5	1.79
4	4.8	22.1	105.7	0.9	2.03
5	14.4	10.6	153.4	1.4	2.19
6	4.8	79.7	385.0	3.4	2.59
7	22.3	31.5	702.2	6.2	2.85
8	30.6	25.3	775.6	6.9	2.89
9	98	31.2	3080.2	27.2	3.49
10	360	16.7	5997.4	53	3.78
Total toxic charge			113308		

<sup>1</sup>  $n (\sum T_i / N)$  in the PEEP formula  
<sup>2</sup> Effluent flow x Toxic Print in the PEEP formula

The results demonstrated a wide range of toxic effects and loadings for textile effluents (Tab. 13). Inter-effluent toxicity differences could be attributed to factors such as: type of industrial process, degree of waste treatment, variability of effluent composition and dilution by process waters. Toxic loads from industries 9 and 10 indicate they contribute the greatest toxic load to the receiving waters; therefore based on PEEP results the first priority would be to reduce the toxic loading from industries 9 and 10.

The PEEP index also allows for monitoring the treatment efficiency of industrial effluents. This is illustrated in Figure 2 following the collection of seven composite effluent samples that were taken before and after treatment from a cosmetic industry. The objective of the waste treatment was to maximize the removal of the toxicants causing the toxic loading. In all cases, application of biological and chemical treatments proved to be beneficial in producing a reduction in toxic loading.

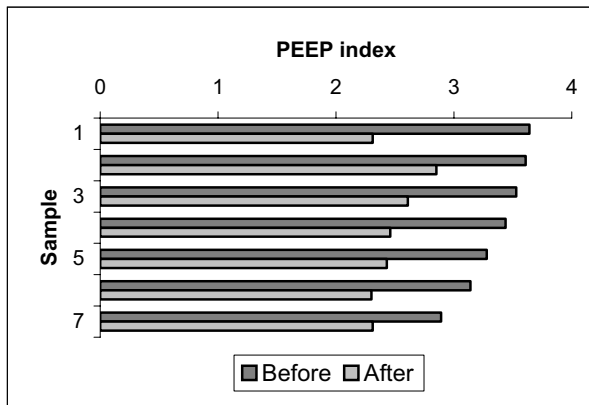


Figure 2. Effect of biological treatment on toxicity reduction of cosmetic industry effluents as indicated by PEEP index values.

In brief, the PEEP index is a useful HAS to apply in comparative studies of wastewater effluents to assess their ecotoxicity and toxic loading. Some of its advantages include the fact that it considers results from different toxicity tests and endpoints, while integrating all possible antagonistic, additive or synergistic interactions that can occur between toxicants in a complex liquid sample. Furthermore, the use of a single PEEP value becomes very useful for decision-makers who are then able to take science-based decisions to prioritize corrective actions on industries whose effluents are the most toxic for the aquatic environment. It is also noteworthy to point out that the PEEP index can be applied anywhere with any number or type of tests and endpoints to suit the needs and expertise of laboratories internationally.

## 6. Conclusions

The battery of test approach for toxicity testing is now a universally-accepted concept. It has recently been applied in Latin American countries and is presently recognized as a critical tool for the assessment of complex mixtures. Interpretation of hazard by reducing complex ecotoxicological data into a single numerical value (e.g., PEEP index) is generally favoured by decision-makers involved in various facets of environmental regulation.

Applying the WaterTox battery of tests in Argentina, Chile and Colombia for toxicity assessment of chemical contaminants present in different types of complex matrices by means of existing, modified or developed HAS approaches has proven to be environmentally beneficial. Water and wastewater samples, sewage sludge and biosolids from municipal treatment plants and effluent toxic loads, as well as pure compounds, were effectively scored as toxic or non-toxic with the ranking systems employed, thereby allowing them to be differentiated in terms of their adverse potential. In all cases, similar bioanalytical tools were employed to conduct these evaluations.

Environmental programs in Chile and Colombia have set clear goals regarding the treatment of effluents and have already initiated research projects in cooperation with academic groups. Argentinean regulatory agencies have also been incorporating tools for the interpretation of results from toxicity testing and categorization of hazardous wastes. There are diverse applications for bioanalytical tools, particularly when they are integrated into testing batteries, as documented in this chapter. While the simple HAS systems described are unquestionably useful to assess and rank toxicity, future refinement will necessitate additional appraisals on types/numbers of tests and endpoints required to maximize detection of toxicity potential and to sharpen their power to discriminate samples based on more subtle levels of toxicity. Cost-efficiency, reliability of testing and ease in interpreting hazard will also be critical in future initiatives designed to integrate HAS in environmental risk assessment processes by local environmental protection agencies.

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## Abbreviations

AS	Activated Sludge
CIMA	Environmental Research Centre
EC20	20 % effect inhibitory concentration
EC50	50 % effect inhibitory concentration
EDAR	effect:dilution average ratio index
HAS	Hazard Assessment Scheme
IC50	50% effect inhibitory concentration
IDRC	International Research Development Centre
LC50	50% effect on survival
LOEC	Lowest Observed Effect Concentration
PEEP	Potential Ecotoxic Effects Probe
SP	Stabilization Pond
TF	Trickling Filter
TU	Toxic Units.