INVITED REVIEW

ECOTOXICOLOGICAL PROBABLE-NO-EFFECT CONCENTRATIONS FOR ELEMENTS RELATED TO NUCLEAR WASTE

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Manuscript received, 21/10/2005; resubmitted, 6/12/2005; accepted, 8/12/2005.

ABSTRACT

The safety of storage and disposal of nuclear waste is assessed not only on the potential radiological consequences, but also on the potential for chemical ecotoxicity. The waste contains many elements, but the potential for chemical toxicity was considered here for antimony (Sb), beryllium (Be), boron (B), cadmium (Cd), chromium (Cr(III) and Cr(VI)), iodine (I), lead (Pb), mercury (Hg, organic and inorganic forms), nickel (Ni), selenium (Se) and technetium (Tc). The endpoints of interest were soil biota other than plants, specifically micro-organisms, mycorrhizal associations and invertebrates, as well as freshwater plants, invertebrates, fish and amphibians. The ideal probable-no-effect concentration (PNEC) was based on several reports of EC25 (the concentration causing 25% reduction from control) for endpoints relevant to population survival and from chronic, realistic exposure studies. Where possible, the PNEC was the 5th percentile of the population of relevant effect concentrations. A tiered search strategy was used to focus on recent and especially sensitive studies, from this over 650 papers were reviewed and considered, and 55 PNEC values proposed. There remains a scarcity to data for certain element and endpoints, whereas others are well represented in the literature.

Key words: PNEC; antimony; beryllium; boron; cadmium; chromium; iodine; lead; mercury; nickel; selenium; technetium.

INTRODUCTION

Many agencies in the world are tasked with deriving guidelines for contaminants in the environment. In effect, these agencies largely access the same scientific literature, but each applies its own methods and logic to the interpretation of this literature. This logic changes with time, and the scientific literature reporting ecotoxicological information is expanding rapidly. The task of deriving guidelines is formidable.

The nuclear industry, especially as related to waste management, is dependent upon ecotoxicological guidelines because these guidelines are most often the basis used to assess a level of impact. However, nuclear waste includes elements not often considered in detail by environmental agencies. The objective of this study was to provide a state-of-the-art derivation of guidelines for elements considered potentially important for nuclear waste. Additionally, because the same interpretive process was used for a number of important elements, a secondary objective was to consider element-to-element commonalities. Only a summary of the findings from the literature is presented here.

METHODS

Ecotoxicological approach

In general, the intent of ecotoxicology is to describe the potential impact of contaminants on ecosystem components so that good decisions can be made to protect the ecology of an environment. With most species, this means protecting the survival of a population. Thus, it is important to protect against effects on growth and reproduction, not just lethality. For some species, such as endangered or highly valued species, the objective may be to protect individuals.

Non-lethal endpoints are preferred, because even at a low level of lethality there may be other effects that are detrimental to survival of a population. Expressed as an effect concentration, an 'EC25' would indicate the concentration at which performance was 25% less than the control. The effect level in this case was 25%, which is used in this study as the preferred quantity. More severe effect levels (e.g. 50%) were considered too great an impact, and lower levels (e.g. 10%) can only rarely be shown to be different statistically from controls.

Literature searching

For some of the elements of concern, notably Cd, Cr, Pb, Hg and Ni, there are many research papers and a history of prior developments of guidelines. In order to avoid duplication of this effort, the search strategy for these was to first identify the literature used previously by other agencies to set guideline values. Then, more recent papers that cited these benchmark papers were identified, obtained and interpreted. It was assumed that papers finding more sensitive endpoints would cite the previous benchmark papers. In addition, general searches of the literature were also done for relevant papers more recent than the benchmark papers. Where the literature was less complete, especially for Sb, Be, I and Tc, the searches were comprehensive, not bounded by date. The remaining elements, B and Se, are well researched for certain endpoints of interest and not for the others, and so the search strategies were modified accordingly.

Data acceptance criteria

Not all literature intended to convey ecotoxicity data is of sufficient quality to consider. Conversely, there are data not specifically intended for ecotoxicology that are useful from

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that perspective. Therefore, acceptance criteria are needed. At the same time, it is recognised that in the absence of directly relevant and fully acceptable data, it is sometimes necessary to consider less-ideal data. We present the criteria here as attributes of an ideal reference.

1. Replicated study or regression-based study with effects that are proven by statistical tests to be different from the negative (zero level) control
2. Monotonic and smooth response to treatment levels
3. Experimental conditions are reasonable and performance of controls is as expected
4. Confounding factors are absent or understood
5. At least three treatment levels, typically log-scaled
6. Original source rather than a review
7. Peer-reviewed source rather than an unpublished report
8. Experimental media are relevant

Selection of endpoint

The preferred endpoints had direct and obvious ecological relevance, and were sought for the following organisms:

Soil

- Microbes, including measurements of enumeration, respiration, endogenous and exogenous enzyme activities, functional activity (e.g. rates of nitrification) and biochemical markers (e.g. ergosterol used to indicate fungal activity).
- Mycorrhizae, as a specific class of microbes that often symbiotically interact with plants and are nearly essential for the survival of certain forest species, usually quantified by enumeration.
- Invertebrates, including earthworms (several genera), pot worms (enchytraeids), nematodes, microarthropods (usually collembolans and mites), and less frequently land snails and soil-dependent insects.

Freshwater

- Plants, including phytoplankton and submergent and emergent macrophytes.
- Invertebrates, including pelagic and benthic organisms from zooplankton to macroinvertebrates, and including ephemeral insect stages.
- Fish, including all vertebrate fin fish through all stages of their life cycle, and not differentiated by trophic level because many species operate at various trophic levels through their life cycle.
- Amphibians, usually frogs or toads but freshwater turtles (amphibious reptiles) may be used instead.

Toxicity to terrestrial plants was not included here. A separate PNEC was not defined for aquatic microbial processes, but data for these were considered in support of the PNEC for aquatic invertebrates.

Many papers will report multiple endpoints; indeed the preference in ecotoxicology is to apply a battery of bioassays because each contaminant will affect one bioassay more than another. In addition, it is common to report several endpoints for a given organism, such as for aquatic invertebrates: hatching success, growth, and reproduction. The approach in this study was as follows:

- If several endpoints for a given bioassay organism were reported, only the most sensitive, ecologically relevant endpoint was considered.
- If several species of the same type of organism, such as a series of carnivorous fish species, were used in one study, only the most sensitive relevant species was considered.
- If different types of organisms were used in one study, such as earthworms and microarthropods as invertebrates in soil, they were considered separately.
- If a range of media characteristics, such as a series of soils, were used, and they had an important effect on the toxicity in a systematic way, they were considered separately. For example, if a study reported toxicity in 5 soils, of which 2 were sands and 3 were clays, and there was a clear difference between the sands and clays, then the results would be considered as two entries, one for sand and one for clay. For the soils where results were considered the same, the geometric mean effect concentration among those soils were used.

Summarisation of information to derive probable-no-effect concentrations

There are various nuances to the process of deriving probable-no-effect concentrations (PNECs) that differ among agencies, but the general procedure is fairly consistent. The first step, described in the previous sections, is to compile a self-consistent database of ecotoxicology information. Consistency in interpretation of the literature for input to the database is important, because the next step requires comparison across the various studies. Once the database is assembled, the next step varies depending on the amount and relevance of the data.

The ideal is to have a large amount of independent and representative effect-concentration data (perhaps at least more than 30 values). From this one would construct a probability frequency distribution (usually lognormal), and the most common is to set the PNEC as the 5^{th} percentile of this distribution. The implication is that 5% of the data, the lowest 5% of the effect concentrations, is either unreliable in some unknown way, or represents especially sensitive organisms that cannot be realistically protected in anything other than pristine environments. Although the choice of 5% is somewhat arbitrary, the attractiveness of the concept is that it recognises that not all organisms can live in all settings, and more importantly it avoids continued revision of the PNEC every time a researcher finds a yet-more-sensitive bioassay. The potentially misleading aspect is that, to fully and appropriately characterise the probability frequency distribution, one would need to include the results from insensitive or outright tolerant bioassays. In fact, the incentives driving ecotoxicology research lead to a strong bias to only report the most sensitive bioassays, causing an inherent downward-skew to the distribution. The 5^{th} percentile (y), assuming a lognormal distribution, is computed from the geometric mean (GM) and geometric standard deviation (GSD) as $y = 10^{-(1.65 \cdot \log(GSD) - \log(GM))}$.

Where there are fewer data, the choice of PNEC is more subjective. In order to benefit from the 5th percentile method, the following can be used for guidance. An ideal data set might have 30 to 100 entries. The GSD for ecotoxicity data is typically at least 3 (i.e. the 5th percentile is at least 9-fold lower than the geometric mean (GM)). The expected values are shown in Table 1.

This implies that if the lowest effect concentration in the database is more than 3-fold lower than the 2nd-lowest, then one could argue it may be lower than the 5th percentile and should be lower than the chosen PNEC. Obviously, this is only guidance and depends on many statistical and ecotoxicological factors, but it does provide some unifying rationale between the 5th percentile method and the more subjective approach required in many cases.

Table 1. Expected positions of 5th percentiles and values next above and below the 5th percentiles, in data sets of different sizes.

Number of data	Approximate rank position of 5th percentile	Next lower value	Next higher value
30	2nd lowest value	2.6-fold lower	1.2-fold higher
100	5th lowest value	1.6-fold lower	1.1-fold higher

RESULTS AND DISCUSSION

Structure

This paper summarizes the findings. For each element, there is a brief description of the literature available. The discussions of the endpoints are ordered as in the list of endpoints above. Where the proposed PNEC are based on a few specific papers, they are individually cited. In other cases, the proposed PNEC is derived as the 5th percentile of an assumed lognormal distribution of many reported effect concentrations, and for the sake of brevity the individual papers are not cited here. If no data were found in the literature for a specific element and endpoint, where possible a PNEC value is suggested from among other PNECs derived for the same element. All proposed values are given in Tables 2 to 15.

Antimony

Antimony primarily exists in the environment in cationic forms, but it also has anionic forms such as antimonates and antimonides. Three papers were found to report toxicity of Sb to soil microbes. The data of Cornfield (1977) allowed estimation of an EC25 at 55 mg kg^{-1} for respiration. Picard and Bosco (2003) found increased auxin-producing bacteria, with an EC25 for the most sensitive stage at 115 mg kg^{-1}. Hammel et al. (1998) investigated three inorganic forms of Sb in three soils, and found no one chemical form was consistently more toxic than another. The lowest EC50 for growth of the soil algae *Chlorococcum infusionum* was 125 mg kg^{-1}. It is proposed that a provisional PNEC for soil microbes be set at 60 mg kg^{-1}, recognising (Table 2) that this is not well supported by the literature and should be considered only for very general guidance.

One paper (Hartley et al. 1999) gave a no-observed-effect concentration (NOEC) for the effect of Sb on mycorrhizae associated with Scots pine of 0.3 mg kg^{-1}. As this is a NOEC, the EC25 is probably higher and it is recommended that the PNEC derived for plants (elsewhere) be used as a provisional PNEC for mycorrhyzal organisms. No data were found on which to derive a PNEC for soil invertebrates.

For freshwater plants, the data of He and Yang (1999) allowed interpolation of an EC25 at about 10 000 µg L^{-1} for germination of rice seed in water, and is taken here to represent a freshwater macrophyte. Hammel et al. (1998) reported EC50 values for green algae *Chlorococcum infusionum* of 43 000 µg L^{-1} and *Scenedesmus subspicatus* of 59 000 µg L^{-1}. These are much higher effect concentrations than those reported for freshwater invertebrates and fish (below), so that it would seem unlikely that the PNEC for freshwater plants will be a critical value in an assessment context. It is proposed that a provisional PNEC for freshwater plants be set at 10 000 µg L^{-1}.

There is a considerable range in effect concentrations for freshwater invertebrates, from an EC05 of 120 µg L^{-1} for the protozoan *Entosiphon sulcatum* (Bringmann and Kühn 1980) to a LC50 of >20 000 µg L^{-1} for the nematode *Caenorhabditis elegans* in water. There are few data, and it is relevant to consider data for other aquatic organisms such as microbes. Aquatic microbes, including *Vibrio fischeri* used in the Microtox chronic toxicity test (Hsieh et al. 2004), the green alga *Chlorococcum infusionum* (Hammel et al. 1998), and the SOS Chromotest (Lantzsch and Gebel 1997) had EC50 or lowest-observed-effect concentration (LOEC) values of 640, 7000 and 43 000 µg L^{-1}, respectively. The GM and GSD of the effect concentrations for freshwater microbes and invertebrates are 6000 µg L^{-1} and 6.9 respectively, giving a 5th percentile of 300 µg L^{-1}. There are few other criteria to select a PNEC. We propose 300 µg L^{-1} as a provisional PNEC for freshwater invertebrates, recognising that this is not well supported by the literature.

The only report of toxicity to freshwater fish was an EC15 of 18 000 µg L^{-1} from growth of larval tilapia (*Oreochromis mossambicus*), by Lin and Hwang (1998). LeBlanc and Dean (1984) reported a NOEC at 7.5 µg L^{-1}, which was the solubility limit of the antimony trioxide they used and therefore not useful. There were no data for amphibians. Given this lack of data, the provisional PNEC of 300 µg L^{-1} for freshwater invertebrates is also proposed for freshwater fish and amphibians.

Beryllium

Beryllium, relatively abundant in the lithosphere, is widely distributed at very low concentrations and binds easily with organic substances. Only one paper (Wilke 1989) was found dealing with the toxicity of Be in soil, but it was from an ideal study in many regards. Soils were contaminated with several concentrations of each of a series of elements and left to incubate for 9 years. Several endpoints were measured, with dehydrogenase and microbial biomass usually the most sensitive. The LOEC for Be was 14 mg kg^{-1}. It is proposed that a provisional PNEC for all soil organisms be set at 10 mg kg^{-1}, recognising that this is not well supported by the literature and should be considered only for very general guidance (Table 3).

No data were found on which to derive a PNEC for freshwater plants or amphibians. Only two papers were found dealing with toxicity of Be in freshwater systems, both for invertebrates. Bringmann and Kühn (1980) reported that Be was selectively toxic to the protozoan *Entosiphon sulcatum*, with an EC05 of 4 µg L^{-1}. Williams and Dusenbery (1990) reported a LC50 of 140 µg L^{-1} for the nematode *Caenorhabditis elegans* in water. Although this is a free-living nematode usually associated with soil, most experiments with it are done in water or agar media, and so the results are more relevant to aquatic systems. For a given endpoint, the EC25 would be between the EC05 and the LC50, and the GM of these two values is 20 µg L^{-1}. Prager (1997) summarized several USEPA sources listing LC50 values of 150 to 20 000 µg L^{-1} for freshwater fish, with lower values for soft water. It is proposed that a provisional PNEC for all freshwater organisms be set at 20 µg L^{-1}, recognising that this is not well supported by the literature and should be considered only for very general guidance.

Boron

Boron is relatively abundant in the lithosphere, and is very mobile but not evenly distributed. Its common valence state is B^{3+} and it exists usually as an anionic borate or boric hydroxide.

Only two studies were found that described toxicity to organisms in soil other than plants. Rogers and Li (1985) provided data so that the EC25 for dehydrogenase activity in soil could be interpolated, giving a value of 180 mg kg^{-1}. Senwo and Tabatabai (1999) used only one concentration of B, and recorded the degree of inhibition of the aspartase enzyme system. At 54 mg kg^{-1}, the effect level was EC43. It is reasonable to expect that had they measured EC25, it would be at an even lower concentration. It is difficult to assign ecological relevance to microbial bioassays (Sheppard 1999), and so it is not possible to derive a definitive PNEC from these data, and they certainly do not represent invertebrates. It is recommended that the PNEC derived for plants be used to protect all soil organisms (Table 4).

Although B is expected to be quite toxic to terrestrial plants, only a few studies reported toxicity to freshwater plants. Bringmann and Kühn (1980) reported a 'toxicity threshold' for green algae at 160 µg L^{-1}, and this was interpreted here as an approximation of an EC05. The same authors reported toxicity thresholds of 280 µg L^{-1} for freshwater protozoans and 290 000 µg L^{-1} for freshwater bacteria (*Pseudomonas*). These values for algae and protozoans are markedly lower than the effect concentrations found for higher aquatic plants, fish or invertebrates, so setting the PNEC at this level will result in it being the lowest freshwater PNEC. The EC25 for rice (included here because it is an emergent macrophyte) was 9000 µg L^{-1} (Powell et al. 1996), and for duckweed was 15 000 µg L^{-1} (Davies et al. 2002). It is proposed that a provisional PNEC for freshwater plants be set at 200 µg L^{-1}, recognising that this is not well supported by the literature and should be considered only for very general guidance.

There were five EC05 or EC25 values found for freshwater invertebrates: 1000, 10 000, 13 000, 14 000 and 17 000 µg L^{-1}. The lowest of these was an EC05 as cited in a review paper by Butterwick et al. (1989), and is tenfold lower than the next highest effect concentration. Because the original data were not available, the effect level was lower than ideal, and there were no supporting data, this was not used as the PNEC. The next series of effect concentrations are relatively close together and are mostly EC25, so the PNEC proposed from these is the lowest of the series: 10 000 µg L^{-1}.

Rowe et al. (1998) noted that water concentrations below 2.2 µg L^{-1} could lead to B deficiency for rainbow trout or zebrafish. This is well below the toxic effect concentrations that were reported to range from 1000 to 356 000 µg L^{-1}. Butterwick et al. (1989) found two papers that reported NOEC at 750 µg L^{-1}, which as a NOEC may be expected to be lower than an EC25-based PNEC. Three papers were found to list LOEC at 1000 µg L^{-1}, and the only paper where EC25 could be estimated (Loewengart 2001) gave the level at 15 000 µg L^{-1}. Usually the LOEC is close to the EC25 because the statistical power of many ecotoxicity experiments is such that less than a 25% impact is not statistically significant. Following this assumption that LOEC is an approximation for EC25, the proposed PNEC for freshwater fish is 1000 µg L^{-1}. No data were found on which to derive a PNEC for amphibians, and so the PNEC for fish is recommended to represent amphibians.

Cadmium

Cadmium is a well-studied element, predominantly present as the Cd^{2+} ion, with various complex ions present in solution. Over 40 papers were found reporting toxicity of Cd to soil microbes, with 20 papers reporting toxicity below 20 mg kg^{-1}. The review paper by Bååth (1989) reported a LOEC of 2 mg kg^{-1}. Smolders et al. (2001) observed an EC14 of 2 mg kg^{-1} in one of four soils, and the corresponding EC50 was 304 mg kg^{-1}. Considering all of the effect concentrations found, the GM and GSD were 40 mg kg^{-1} and 8.2, implying a 5th percentile of 1.3 mg kg^{-1}. This seems consistent with the review by Bååth (1989) and the results of Smolders et al. (2001), and so the proposed PNEC for soil microbial activity is 2 mg kg^{-1} (Table 5).

There were six papers reporting effects on mycorrhizae, all in the relatively narrow range of 2 to 10 mg kg^{-1}. These values are consistent with the PNEC proposed for other soil microbial effects, and so the PNEC proposed for mycorrhizae is the same as that for other microbial processes, 2 mg kg^{-1}.

As with the soil microbial data, a large number of studies have reported on toxicity to soil invertebrates. The most sensitive had an exceptionally low effect concentration (Callahan et al. 1994), but results from this paper for other elements were also exceptionally low, and were not considered for the other PNECs. The progression of effect concentrations among the other papers was quite gradual, suggesting considerable confidence in proposing a PNEC value. Excluding the data of Callahan et al. (1994), the GM and GSD were 26 mg kg^{-1} and

Table 2. Proposed PNEC for antimony.

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})		1	20-40[a]		
microbial	60			low	
mycorrhizae	nd				Use PNEC for plants or microbes
invertebrates	nd				Use PNEC for microbes
Aquatic (µg L^{-1})		0.2			
plant	10000			low	
invertebrates	300			low	
fish	300			low	Based on invertebrates
amphibians	300			low	Based on invertebrates

[a] Canada (CCME 2004)

Table 3. Proposed PNEC for beryllium.

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})		15[a]	4-8[b]		
all	10			low	Based on microbial endpoints
Aquatic (µg L^{-1})		<0.001	100[b]		
plant	nd				Use PNEC for invertebrates
invertebrates	20			low	
fish	20			low	Based on invertebrates
amphibians	nd				Use PNEC for invertebrates

[a] Upper 95th percentile in Canada 1.9 mg kg^{-1} (unpublished)
[b] Canada (CCME 2004)

Table 4. Proposed PNEC for boron.

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})		80			
all	nd		2[a]		Boron quite toxic to plants, so PNEC for plants recommended for all soil biota
Aquatic (µg L^{-1})		10	370[b]		
plant	200			moderate	
invertebrates	10 000			moderate	
fish	1000			moderate	
amphibians	nd				Use PNEC for plants, given that many amphibians rely on plants

[a] Canada (CCME 2004)
[b] Australia (ANZECC 2000)

6.3, implying a 5th percentile of 1.2 mg kg^{-1}. This is consistent with the EC25 from Schmidt et al. (1991) and is close to the PNEC set for soil microbial processes. The proposed PNEC for soil invertebrates is 1.2 mg kg^{-1}.

There were over 20 papers dealing with toxicity to aquatic plants. The effect levels vary among these papers, but the progression of effect concentrations is rather gradual, which supports identifying a PNEC. However, all of these are with micro-plants, usually algae, and none represent macrophytes. The overall GM and GSD were 56 µg kg^{-1} and 10, implying a 5th percentile of 1.2 µg L^{-1}. There was only one effect concentration recorded below this, but there are no obvious outliers among the data. With a more typical GSD of 4, the 5th percentile would be 6 µg L^{-1}. Because there were no data for other types of plants, a lower PNEC may be prudent. The proposed PNEC is the computed 5th percentile of 1.2 µg L^{-1}.

There were about 100 papers citing effects on freshwater invertebrates, with effect concentrations ranging from 0.003 to 940 000 µg L^{-1}. However, both these extreme values are not supported by other literature, and so were considered outliers and were ignored. Several papers reported effect concentrations between 0.1 and 0.3 µg L^{-1}, and generally for appropriate effect levels, suggesting that this may be the appropriate range for the PNEC. Twelve studies reported on effects on aquatic microbial processes, but with a range of effect concentrations from 2.9 to 9000 µg L^{-1}, they did not influence the choice of PNEC for invertebrates. The overall GM and GSD for aquatic invertebrates were 16 µg kg^{-1} and 23, implying a 5th percentile of 0.087 µg L^{-1}. This is an exceptionally large GSD. With a more typical GSD of 4, the 5th percentile would be 1.6 µg L^{-1}. However, the former value is more consistent with the papers listed below (Table 6), and these papers are good representations of the ideal endpoints. The proposed PNEC is 0.2 µg L^{-1}, based on the papers listed in Table 6.

Toxicity of Cd to freshwater fish has been the subject of many studies, and several review papers are available. The review papers (Spry and Wiener 1991; Scott and Sloman 2004; EPA 2001) suggest the guidelines should be about 0.5 µg L^{-1}. Only one study (Rombough and Garside 1982 cited by CEPA 1994) was found that had a lower effect concentration of 0.47 µg L^{-1}, and this was an EC12 which would be expected to be lower than the desired EC25. An interesting attribute of the 20 most-sensitive papers was the non-lethal endpoints, including a number of behavioural endpoints. These suggest that the research has evolved to the stage of examining very subtle effects.

Roux et al. (1996) proposed guidelines of 0.15 µg L^{-1} in soft water (<60 mg L^{-1}) and 0.34 for hard water (>180 mg L^{-1}). These were generic for all aquatic organisms relevant to South Africa. It is quite certain that hardness is important to Cd toxicity, and the 20 most-sensitive studies were in soft water.

The overall GM and GSD for fish, excluding two values that were above 100 000 µg L^{-1}, were 11 µg L^{-1} and 15, implying a 5th percentile of 0.13 µg L^{-1}. This is an exceptionally large GSD. With a more typical GSD of 4, the 5th percentile would be 1.1 µg L^{-1}. In this case, because several review papers have suggested a guideline of 0.5 µg L^{-1}, and this lies between our two estimates of the 5th percentile, it is proposed the PNEC be 0.5 µg L^{-1}. This is intended to be protective in all water chemistries, a higher PNEC possibly could be defined for hard water.

There were eight studies that dealt with freshwater amphibians, and the lowest effect concentration was for an acute LC50 in African clawed frog at 0.2 µg L^{-1}. This suggests greater sensitivity than for fish. However, all the other effect concentrations were much higher. James and Little (2003) reported hormesis at 5 µg L^{-1}, which suggests a deleterious effect would be at a higher concentration. The remaining effect concentrations were over 200 µg L^{-1}. The overall GM and GSD for amphibians were 120 µg kg^{-1} and 22, implying a 5th percentile of 0.7 µg L^{-1}. This would be consistent with the PNEC proposed for fish. However, this is an exceptionally large GSD. With a more typical GSD of 4, the 5th percentile would be 12 µg L^{-1}. Given there was only one study indicating sensitivity greater than for fish, it is proposed the PNEC be 12 µg L^{-1}.

Chromium (III)

Chromium (III) and (VI) are handled separately because they are very different in ecotoxicological effect, with Cr (III) less soluble and less toxic than Cr (VI). Not all papers indicate which chemical species was used. Additionally, once added to soils or water, the chemical speciation will change, as there is an equilibrium distribution established fairly quickly. Thus, the actual toxic species may not be known. In general, Cr(VI) seems to be more commonly used in aquatic ecotoxicology and Cr(III) in soil ecotoxicology, but rarely is the speciation confirmed during or after the bioassay. In soils, Cr(III) tends to be the predominant species, but Cr(VI) may be so much more toxic that even a small fraction of the total Cr as Cr(VI) may dominate the toxicity response. The approach here was to differentiate the papers that clearly dealt with either Cr (III) or Cr(VI), and include the unspecified papers with both groups.

The range in effect concentrations for soil microbial processes was from 1 mg kg^{-1} (an EC60) to 260 mg kg^{-1} (an EC39). There are no obvious outliers in this group of data. The GM and GSD of the effect concentrations for soil microbes are 61 mg kg^{-1} and 5.4, giving a 5th percentile of 3.8 mg kg^{-1}. Only a study by Zibilske and Wagner (1982) had a lower effect concentration, and the next highest was 10 mg kg^{-1} from Rogers and Li (1985). It is proposed the PNEC be 4 mg kg^{-1}, recognising that it is only moderately well supported by the available literature. No data were found on which to derive a PNEC for mycorrhizae, and so the PNEC for plants or soil microbes is recommended to represent mycorrhizae (Table 7).

Only two studies were found that reported effect on soil invertebrates, both for reproduction in *Eisenia andrei.* An EC25 of 57 mg kg^{-1} was interpolated from van Gestel et al. (1992), and an EC25 of 70 mg kg^{-1} from van Gestel et al. (1993). These are from the same laboratory with very similar

Table 5. Proposed PNEC for cadmium.

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})		3[a]	0.9[b]		
			1.4-22[c]		
microbial	2			good	Approaching upper levels of background
mycorrhizae	2			good	
invertebrates	1.2			good	
Aquatic (µg L^{-1})		0.2	0.21[b]		INERIS guidelines are 0.21 µg L^{-1} for soft water (<50 g $CaCO_3$ L^{-1}) and 0.75 µg L^{-1} for hard water
			0.017[c]		
			0.2[d]		
plant	1.2			good	
invertebrates	0.1			good	
fish	0.5			good	Approaching upper levels of background
amphibians	12			good	

[a] Upper 95th percentile in Canada 0.92 mg kg^{-1} (unpublished)
[b] France (GRNC 2002 and INERIS 2004)
[c] Canada (CCME 2004)
[d] Australia (ANZECC 2000)

Table 6. References used as basis for PNEC for cadmium in freshwater invertebrates.

Reference	Effect level	Effect concentration (µg L^{-1})	Organism
Braginskly and Shcherban 1978 cited by EPA 2001	LC50	0.1	*Daphnia magna*
van Leeuwen et al. 1985	EC25	0.15	*Daphnia magna*
Dave et al. 1981	EC16	0.17	*Daphnia magna*
Biesinger and Christensen 1972 cited by CEPA 1994	EC16	0.17	*Daphnia magna*
Borgmann et al. 2004	LC25	0.2	*Hyalella azteca*
Elnabarawy et al. 1986 cited by Vega et al. 1997	LOEC	0.2	*Daphnia pulex*
Lawrence and Holoka 1991 cited by CEPA 1994	EC39	0.2	*Daphnia galeata mendotae* and *Holopedium gibberum*
Hatakeyama and Yasuno 1981 cited by EPA 2001	LOEC	0.2	Cladoceran *Moina macrocopa*
Elnabarawy et al. 1986 cited by Vega et al. 1997	NOEC	0.25	*Ceriodaphnia reticulata*

Table 7. Proposed PNEC for chromium (III).

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})		1000[a]			
microbial	4		3.2[b] 64-87[c]	good	Background range cited as 4 to 1000 mg kg^{-1}, so PNEC potentially within range of background concentrations
mycorrhizae	nd				Use PNEC for plants or microbes
invertebrates	60			low	
Aquatic (µg L^{-1})		0.2	4.7[b] 8.9-56[c]		
plant	nd				Use PNEC for fish
invertebrates	1000			low	
fish	100			low	
amphibians	nd				Use PNEC for fish

[a] Upper 95th percentile in Canada 110 mg kg^{-1} (unpublished)
[b] France (INERIS 2004)
[c] Canada (CCME 2004)
[d] Australia (ANZECC 2000)

endpoints, so it is not surprising the two EC25 values are so similar. It is proposed the PNEC be 60 mg kg^{-1}, recognising that this is not supported by much research.

No data were found on which to derive a PNEC for freshwater plants, and so the PNEC for other freshwater biota is recommended to represent plants. Only two studies reported on effects on freshwater invertebrates, and neither were ideal effect levels. Kühn et al. (1989) indicated a NOEC at 700 µg L^{-1} for reproduction in *Daphnia magna* and Perez-Legaspi and Rico-Martinez (2003) indicated a LOEC at 1000 µg L^{-1} for enzyme inhibition in rotifers. There were also two studies for aquatic microbial processes. Jung et al. (1996) reported an EC50 of 5000 µg L^{-1} for inhibition of beta-galactosidase and McCloskey et al. (1996) reported an EC50 at 9600 µg L^{-1} for microbial (Microtox) assays. Collectively, these suggest that Cr(III) is not especially toxic to aquatic invertebrates and microbial processes. This tolerance to Cr(III) may be because it is not very soluble in water, and this may impede uptake. The proposed PNEC is 1000 µg L^{-1}, noting that is very speculative and is not based on much literature.

Only two studies were found dealing with toxicity to freshwater fish. Stevens and Chapman (1984) provided data to interpolate an EC25 for hatching eggs of steelhead trout (*Oncorhynchus mykiss*) of 110 µg L^{-1}. Pickering and Henderson (1966) studied several fish species in hard and soft waters, and for soft water (20 mg $CaCO_3$ L^{-1}) the LC50 was 3700 µg L^{-1}. In contrast, Roux et al. (1996) reviewed the literature and proposed a guideline of 24 µg L^{-1} to protect fish in freshwater in South Africa. Values proposed by Roux et al. for other elements also stood out as especially low. Given the lack of data, the proposed PNEC is 100 µg L^{-1}, based on the data of Stevens and Chapman (1984) and recognising that this is not well founded in the literature. No data were found on which to derive a PNEC for amphibians, and so the PNEC for fish is recommended to represent amphibians.

Chromium (VI)

For Cr(VI), the effect concentrations for soil microbes were as low as an EC50 of 1 mg kg^{-1} for ammonium oxidase (Rudel et al. 2001), 3.3 mg kg^{-1} for denitrification (Speir et al. 1995) and 3.6 mg kg^{-1} for arylsulfatase activity (Haanstra and Doelman 1991). The GM and GSD of the effect concentrations for soil microbes were 36 mg kg^{-1} and 8.8, with an implied 5th percentile of 1 mg kg^{-1}, which coincides with the lowest of the reported effect concentrations. Because several authors reported EC50 in this range, it seems a credible value. It is proposed that the PNEC for Cr(VI) and soil microbes be set at 1 mg kg^{-1}, recognising that there is a very broad range of values in the literature and there is probably more uncertainty associated with this value than with most PNEC values. No data were found on which to derive a PNEC for mycorrhizae, and so the PNEC for plants or soil microbes is recommended to represent mycorrhizae (Table 8).

Only three papers were found reporting Cr(VI) toxicity to soil invertebrates, and the range in effect concentrations was from 5 mg kg^{-1} for *Eisenia fetida* (Rudel et al. 2001) to 34 000 mg kg^{-1} for protozoan (Berthold and Jakl 2002). It is proposed that the PNEC for Cr(VI) and soil invertebrates be set at 5 mg kg^{-1}, recognising that this is not well supported in the literature, but also that the performance of soil invertebrates will be dependent to some extent on the performance of soil microbes.

The five lowest aquatic plant effect concentrations were relatively consistent: an EC10 of 32 µg L^{-1} (green alga *Scenedesmus subspicatus*), an EC25 of 100 µg L^{-1} (duckweed

Lemna pauciostata), a NOEC of 110 µg L^{-1} (alga and duckweed), an EC50 of 190 µg L^{-1} (microalgae *Scenedesmus quadricauda*), and an EC25 of 260 µg L^{-1} (water lily *Nymphaea alba*). Summarising all the data, the GM and GSD were 280 µg L^{-1} and 2.9, implying a 5th percentile of 48 µg L^{-1}, between the lowest and second lowest recorded effect concentrations. The progression of effect concentrations, generally less than twofold apart at the lower levels, suggests the distribution of possible ecotoxicity results has been reasonably well sampled, so the proposed PNEC is at the observed 5th percentile (with rounding) of 50 µg L^{-1}.

For freshwater invertebrates, the four lowest effect concentrations were very similar, with a LOEC/NOEC (the authors reported the geometric mean of their LOEC and NOEC observations) of 5 µg L^{-1} (*Ceriodaphnia dubia*), a LOEC/EC80 of 10 µg L^{-1} (*Daphnia magna*), a LC50 of 16 µg L^{-1} (*Ceriodaphnia dubia*), and a NOEC of 18 µg L^{-1} (*Daphnia magna*). The other effect concentrations ranged upward to an EC25 of 66 000 µg L^{-1}. Freshwater microbial bioassays were also reported, and their effect concentrations ranged from 380 to 23 000 µg L^{-1}. Among the invertebrate effect concentrations, the GM and GSD were 120 µg L^{-1} and 6.4, with an implied 5th percentile of 6 µg L^{-1}. Given that the study reporting 5 µg L^{-1} (Hickey 1989) seems to be well done, and that two of the other four lowest effect concentrations were for more severe effect levels than EC25 (i.e. the EC25 would be lower than the values recorded here), the proposed PNEC is 5 µg L^{-1}.

The effect concentrations for fish span a very broad range, from an NOEC of 1000 µg L^{-1} for rainbow trout (*Oncorhynchus mykiss*) to 38 000 µg L^{-1} for striped bass (*Morone saxatilis*), and higher for two other papers given less credibility. The most sensitive study (Van Der Putte et al. 1982) appears to have been well done, the NOEC of 1000 µg L^{-1} was based on behaviour (coughing) and the LOEC of 2000 µg L^{-1} on ventilation rate. The next most sensitive study gave an EC25, the desired effect level, and included second-generation effects, an unusual but very relevant endpoint. The GM and GSD of all the recorded values were 16 000 µg L^{-1} and 4.6, with an implied 5th percentile of 1300 µg L^{-1}. This is between the two lowest recorded effect concentrations, and seems an appropriate level given the endpoints of these two studies. It is proposed that the PNEC be set at 1300 µg L^{-1}.

Slooff and Canton (1983) investigated the toxicity of Cr(VI) to the African clawed frog (*Xenopus laevis*), a common amphibian bioassay organism. The NOEC for mortality was 350 µg L^{-1}, and surprisingly the NOEC for effects on growth and development were higher, at 1100 µg L^{-1}. No other data were found for amphibians. This value of 350 µg L^{-1} is substantially lower than the PNEC proposed for fish and substantially higher than that proposed for invertebrates, but it is relevant to note that Slooff and Canton (1983) also studied both fish and invertebrates, and found that the amphibian was indeed intermediate. This lends credence to the value despite there being no other study with amphibians, and it is proposed that the freshwater PNEC for Cr(VI) and amphibians be set at 350 µg L^{-1}.

Iodine

Ecotoxicity of I has not been well widely researched, probably because iodine is not an important emission from most industrial activities. It has been considered from the viewpoint of nuclear waste management, because the radionuclide ^{129}I has such a long half-life that the chemical toxicity of iodine may exceed in importance the radiological toxicity of ^{129}I (Sheppard and Evenden 1995; Laverock et al. 1995).

Three studies addressed the toxicity of I to soil microbial processes. Lewis and Powers (1941 cited by Sheppard and Evenden 1995) examined effects on *Azotobacter* in suspension culture and noted a NOEC for nitrogen fixation at 50 mg kg^{-1}. Sheppard and Hawkins (1995) examined the effect of I on several microbial assays and their data suggested an EC25 in suspension between 75 and 200 µg L^{-1}. Using the sorption data from a related experiment (Sheppard et al. 1989), this implies an EC25 in soil of 480 mg kg^{-1}. However, this is for a peat soil. Medeiros and Rocha (1969) seemed to indicate a NOEC at 1000 mg kg^{-1} for fungi. None of these data are directly relevant for PNEC in mineral soils. Only Sheppard and Hawkins (1995) indicated an effect. Assuming a tenfold difference in soil bulk density between an organic and mineral soil, a useful approximation may be to project from their data an EC25 at 50 mg kg^{-1} in mineral soils.

All the information for soil invertebrates comes from the same laboratory (Sheppard and Evenden 1994, 1995). The lowest EC25 of 5.8 mg kg^{-1} was not strictly a soil bioassay (Sheppard and Evenden 1995). The soil pore water was extracted and an aquatic bioassay with *Daphnia magna* was applied. The same study also reported ecotoxicity for microarthropods with an EC25 of 25 mg kg^{-1} and a NOEC for earthworms at 1000 mg kg^{-1}. The proposed PNEC is 25 mg kg^{-1}, based on both the invertebrate (microarthropod) results from Sheppard and Evenden (1995) and supported by the extrapolation to mineral soils from the microbial results of Sheppard and Hawkins (1995). The PNEC for I are summarized in Table 9.

Laverock et al. (1995) did a very thorough investigation of toxicity of I to *Daphnia magna* and trout *Oncorhynchus mykiss,* considering the effects of I speciation, water hardness, water chloride content and total organic carbon content. Here, the results for I_2 are excluded as this is not a relevant chemical species for nuclear waste management scenarios. The lowest LC50 for *Daphnia* was 170 µg L^{-1} and for trout was 220 000 µg L^{-1}. Sheppard and Hawkins (1995) reported toxicity to microbial processes in soil pore waters, and their data suggest an EC25 at 160 000 µg L^{-1}. The only other relevant study was by Bringmann and Kühn (1980), where for *Scenedesmus quadricauda* (green alga), their data suggest an EC05 at 40 000 µg L^{-1}. The outcome of these studies is that I is not very toxic to aquatic organisms. The LC50 for *Daphnia* is the lowest found, and as a LC50 it is assumed that a non-lethal EC25 would be lower. The proposed PNEC is 100 µg L^{-1}, recognising that this is poorly supported by the literature.

Lead

Lead is one of the least mobile of the so-called heavy metals. The effect of Pb on soil microbial processes is particularly well researched, probably because these endpoints have consistently been shown to be more sensitive than others for Pb. The lowest three effect concentrations were reasonably similar: an EC25 of 40 mg kg^{-1} (arylsulfatase and acid phosphatase), an EC25 of 50 mg kg^{-1} (N mineralisation), and a LOEC of 100 mg kg^{-1} (respiration in litter). The remaining effect concentrations ranged upward to an EC25 of 10 000 mg kg^{-1} (respiration). All three of these low effect concentrations are valid but have drawbacks for their interpretation. The overall GM and GSD for all the soil microbe effect concentrations were 540 mg kg^{-1} and 3.3, with an implied 5th percentile of 76 mg kg^{-1}. This is slightly higher than the two lowest effect concentrations (Effron et al. 2004; Chang and Broadbent 1982), and because both are credible studies with mineral soils, it is proposed that the PNEC be set closer to these two studies, at 50 mg kg^{-1}. No data were found on which to derive a PNEC for mycorrhizae, and so the PNEC for plants or soil microbes is recommended to represent mycorrhizae (Table 10).

The lowest two effect concentrations for invertebrates in soil are much lower than all the remaining values. There is no obvious reason why the effect concentration reported by Callahan et al. (1994) for four earthworm species was so low; they reported a LC50 of 0.0056 mg kg^{-1}. Several of the lowest recorded effect concentrations were NOEC, and so may not have accurately represented an effect. However, there was a consistent group of effect concentrations ranging from 40 to 63 mg kg^{-1}, and the consistency would make it logical that the PNEC would fall near this range. The overall GM and GSD of the effect concentrations, excluding Callahan et al. (1994), were 410 mg kg^{-1} and 4.6, with an implied 5th percentile of 32 mg kg^{-1}. The proposed PNEC for soil invertebrates is based on the 5th percentile at 30 mg kg^{-1}.

The four lowest effect concentrations for freshwater plants were based on growth of the alga *Selenastrum capricornutum* (now known as *Pseudokirchneriella subcapitata*), and ranged from 104 to 350 μg L^{-1} (Capelo et al. 1993; Christensen et al. 1979; Chen et al. 1997; Wong et al. 2001). In contrast, the next three lowest effect concentrations were all about 2000-3000 μg L^{-1}, all related to photosynthetic activity or growth of algae (Devi Prasad and Devi Prasad 1982; Pawlik-Skowronska 2002; Starodub et al. 1987). The overall GM and GSD of the effect concentrations was 70 μg L^{-1} and 5.6, with an implied 5th percentile of 70 μg L^{-1}. This is below the lowest recorded effect concentration, and this extrapolation may not be warranted. The proposed PNEC is set at the lowest recorded EC25, at 100 μg L^{-1}.

The sequence of effect concentrations for freshwater invertebrates was the gradual progression considered ideal for ecotoxicology data. The specific endpoints range from biomarkers to survival. Some of the similarity among the effect concentrations may be because several involve *Hyalella azteca* and the Borgmann research group (e.g. Borgmann et al. 2004). None the less, these are quite credible values. The highest two effect concentrations were 219 000 and 1 x 10^{6} μg L^{-1}, very much higher than all the intervening effect concentrations. The overall GM and GSD of the effect concentrations, excluding the values above 6000 μg L^{-1} as extreme, were 100 μg L^{-1} and 9.6, with an implied 5th percentile of 2 μg L^{-1}. This is quite low considering the recorded effect concentrations, and results from the large GSD reflecting the very broad range of effect concentrations.

The effect concentrations for aquatic microbial systems are: an EC50 of 180 μg L^{-1} (Microtox), an EC50 of 316 μg L^{-1} (bioluminescent *Escherichia coli*) and a LOEC of 620 μg L^{-1} (sulfate-reducing bacteria *Desulfovibrio desulfuricans*). These are considerably higher than the effect concentrations reported for invertebrates, and so do not create an argument for a lower PNEC for invertebrates.

The choice of PNEC from these values requires some judgement. The 5th percentile value of 2 μg L^{-1} is lower than all but one of the recorded effect concentrations, and is driven this low because there are some high effect concentrations that increased the GSD. With a GSD of 4, which is more typical of effect concentrations, the 5th percentile would have been about 10 μg L^{-1}. Therefore, the proposed PNEC is 10 μg L^{-1}.

As with the freshwater invertebrates, there is a long progressive series of effect concentrations for freshwater fish. The overall GM and GSD, excluding an extreme value of 4.7 x 10^{5} μg L^{-1}, were 300 μg L^{-1} and 18, implying a 5th percentile of 2 μg L^{-1}. As with the freshwater invertebrates, this very low value resulted from the large GSD. With a more typical GSD of 4, the 5th percentile would have been 50 μg L^{-1}, but this is higher than four of the recorded effect concentrations. In this case, the PNEC should reflect the lower effect concentrations reported, so the proposed PNEC is 5 μg L^{-1}. No data were found on which to derive a PNEC for amphibians, and so the PNEC for fish is recommended to represent amphibians.

Mercury (inorganic)

The most toxic forms of Hg are volatile and methylated. Interestingly, Se and Hg interact to be highly protective against toxicity of each other (Cuvin-Aralar and Furness 1991). Because organic species have very different toxicity from inorganic species, the two are differentiated here. Obviously, there are a number of possible organic species, whereas the inorganic form is almost exclusively found as the divalent cation.

There has been considerable research done on toxicity of inorganic Hg to soil microbial processes. The recorded effect concentrations ranged from 0.06 to 1000 mg kg^{-1}. The lowest, 0.06 mg kg^{-1}, was a LOEC (von Stadelmann and Santschi-Fuhrimann 1987 cited by Lindqvist 1991). Sheppard et al. (1993) computed an EC20 from the data of von Stadelmann and Santschi-Fuhrimann (1987) at 1.3 mg kg^{-1}. The overall GM and GSD were 14 mg kg^{-1} and 21, implying a 5th percentile of 0.09 μg L^{-1}. This relatively low value resulted from the large GSD. With a more typical GSD of 4, the 5th percentile would have been 1.4 mg kg^{-1}. The study of Landa and Fang (1978) measured CO_2 evolution from five soils, and noted effect levels of EC10 to EC86 at 0.1 mg kg^{-1}, which supports the 5th percentile based on the full GSD.

Table 8. Proposed PNEC for chromium (VI).

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})			0.035[a]		
			1.0-1.5[b]		
microbial	1			good	
mycorrhizae	nd				Use PNEC for plants or microbes
invertebrates	5			low	
Aquatic (µg L^{-1})			1.0[c]		
			4.1[a]		
			0.4-1.4[b]		
plant	50			good	
invertebrates	5			good	
fish	1300			good	
amphibians	350			low	

France (INERIS 2004)
Canada (CCME 2004)
Australia (ANZECC 2000)

Table 9. Proposed PNEC for iodine.

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})		40			
all	25			low	Background range cited as 0.1 to 40 mg kg^{-1}, PNEC will usually be above background
Aquatic (µg L^{-1})		3			
all	100			low	

Table 10. Proposed PNEC for lead.

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})		200[a]	12[b]		
			70-600[c]		
microbial	50			good	
mycorrhizae	nd				Use PNEC for plants or microbes
invertebrates	30			good	
Aquatic (µg L^{-1})		6	5[b]		
			1-7[c]		
			3.4[d]		
plant	100			good	
invertebrates	10			good	
fish	5			good	Background range cited as 0.01 to 6 µg L^{-1}, PNEC will usually be above background
amphibians	nd			low	Use PNEC for fish

[a] Upper 95th percentile in Canada 31 mg kg^{-1} (unpublished)
[b] France (INERIS 2004)
[c] Canada (CCME 2004)
[d] Australia (ANZECC 2000)

Table 11. Proposed PNEC for inorganic mercury.

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})		1	27[a] 6.6-50[b]		
microbial	0.1			good	Background range cited as 0.01 to 1 mg kg^{-1}, PNEC will usually be above background
mycorrhizae	nd				Use PNEC for plants or microbes
invertebrates	1			moderate	
Aquatic (µg L^{-1})		6	0.24[a] 0.026[b] 0.6[c]		
plant	25			good	
invertebrates	0.5			good	Background cited to range <0.1 to 6 µg L^{-1}, PNEC will usually be above background
fish	0.3			good	
amphibians	0.75			good	

[a] France (INERIS 2004)
[b] Canada (CCME 2004)
[c] Australia (ANZECC 2000)

The proposed PNEC is 0.1 mg kg^{-1}. No data were found on which to derive a PNEC for mycorrhizae, and so the PNEC for plants or soil microbes is recommended to represent mycorrhizae (Table 11).

Effect concentrations for soil invertebrates ranged from 0.12 to 74 mg kg^{-1}, although several were multiple reports from the same laboratories. The lowest effect concentration was an EC25 (Schmidt et al. 1991) of 0.4 mg kg^{-1}. This laboratory also reported the third lowest effect concentration. In each case, this was for egg laying by grasshoppers, a legitimate but unusual soil ecotoxicity endpoint. Sheppard et al. (1993) and Sheppard and Evenden (1994) also reported effect concentrations for earthworms and microarthropods tested in whole soil and *Daphnia magna* tested in soil pore water extracts from contaminated soils. The most sensitive of these was an EC25 of 2 mg kg^{-1} for *D. magna*, but this is a rather indirect assay of soil ecotoxicology because it relies on an aquatic organism. The effect concentrations increased gradually from this level, suggesting there is fair basis for setting a PNEC. The overall GM and GSD were 4.8 mg kg^{-1} and 6.8, implying a 5th percentile of 0.2 mg L^{-1}. None of the recorded effect concentrations were this low. Those based on Schmidt et al. (1991) and Schmidt (1986) were insects that are ephemeral to soil, and the lowest effect concentration from Sheppard et al. (1993) was for an aquatic organism. The lowest EC25 of relevance is for earthworm survival at 16 mg kg^{-1}. Given these uncertainties in the interpretations, the proposed PNEC is 1 mg kg^{-1}.

There is a lot of research on toxic effects with aquatic organisms, undoubtedly related to the fact that environmental impacts of Hg are typically associated with aquatic systems. Among these were seven studies reporting effects on aquatic plants. The effect concentrations ranged from 27 to 800 µg L^{-1}, a relatively narrow range for ecotoxicology effects. The overall GM and GSD were 170 µg kg^{-1} and 3.4, implying a 5th percentile of 23 µg L^{-1}. This is very consistent with the lowest effect concentration, which was an EC50 for the green alga *Selenastrum capricornutum* (now known as *Pseudokirchneriella subcapitata*). The proposed PNEC is 25 µg L^{-1}.

The range in effect concentrations for freshwater invertebrates was from 0.001 to 14 000 µg L^{-1}, a 14-million-fold range. The overall GM and GSD, excluding the values above 1000 µg L^{-1}, were 4.6 µg L^{-1} and 17, implying a 5th percentile of 0.042 µg L^{-1}. This very low value may have resulted from the large GSD. With a more typical GSD of 4, the 5th percentile would have been 0.46 µg L^{-1}. The three lowest effect concentrations were 0.001 µg L^{-1} (esterase inhibition in rotifers), 0.002 µg L^{-1} (survival of decapod *Fasonella clypeata*) and 0.02 µg L^{-1} (survival of *F. clypeata*), all lower than the initial 5th percentile. The lowest effect concentration with a common test species is an EC30 for *Daphnia magna* at 0.69 µg L^{-1}, more consistent with the 5th percentile using a GSD of 4. It is proposed the PNEC be 0.5 µg L^{-1}.

The lowest effect concentration noted for freshwater fish, 0.074 µg L^{-1}, was a LOEC for non-specific cellular immune responses (phagocytosis, respiratory burst, and lymphoblastic proliferation) in rainbow trout (*Oncorhynchus mykiss*) by Sanchez-Dardon et al. (1999). This is an ideal endpoint in that it is non-lethal and a probable harbinger of other effects, but it is difficult to interpret directly in an ecological sense. These effects may not impede populations in settings that are otherwise not a challenge. However, the next lowest few effect concentrations had direct relevance to ecological performance and population survival. The overall GM and GSD were 8.1 µg L^{-1} and 12, implying a 5^{th} percentile of 0.14 µg L^{-1}. This is lower than all but the effect concentration reported by Sanchez-Dardon et al. (1999). There are several reports of effect concentrations at 0.3 µg L^{-1}, and it is proposed that this be the PNEC.

There were five papers reporting effect concentrations for amphibians, with a range of an EC25 at 0.75 to a LC50 at 74 µg L^{-1}. Papers by Birge et al. (1983) and Slooff et al. (1983) reported multiple endpoints for multiple species, and included frogs and toads. The overall GM and GSD were 11 µg L^{-1} and 9.2, implying a 5^{th} percentile of 0.28 µg L^{-1}, a value lower than any of the reported effect concentrations. With a more typical GSD of 4, the 5^{th} percentile would be 1 µg L^{-1}. It is proposed that the PNEC be 0.75 µg L^{-1}, based on the paper by Birge et al. (1983) dealing with an EC25 for hatching success.

Mercury (organic)

Only two studies were recorded that dealt specifically with the effects of organic species of Hg on soil organisms. Beyer et al. (1985) used methyl mercury with *Eisenia foetida* and measured survival and segment regeneration after excision. The EC25 was 5 mg kg^{-1}. Bremner and Douglas (1971) investigated the effects of 100 compounds on the urease activity in three soils, including sodium p-chloromercuribenzoate. The EC32 was 28 mg Hg kg^{-1}. These results are very different in the type of endpoint. A tentative PNEC could be proposed at 5 mg kg^{-1}, but a better situation would be if an appropriate PNEC from effects on plants were consistent with this.

There were two effect concentrations for aquatic plants, an EC50 of 2 µg L^{-1} for methyl mercury and green algae, and a LOEC of 150 µg L^{-1} for methyl mercury and pond weed. There were two effect concentrations for aquatic invertebrates, an EC25 of 0.03 µg L^{-1} for methyl mercury and *Daphnia magna*, and an EC25 of 0.11 µg L^{-1} for methyl mercury and *Daphnia pulex*. There was one paper dealing with aquatic microbial processes, suggesting an EC25 of 30 µg L^{-1} for the ciliate *Tetrahymena pyriformis*. This diverse group of papers is not ideal for setting PNEC, and based on them a tentative PNEC for freshwater biota other than fish of 0.03 µg L^{-1} is proposed (Table 12).

There is more literature for effects of organic mercury on fish. The range of effect concentrations recorded was quite broad, from 0.0045 to 75 µg L^{-1}. The lowest was for hatching success of walleye (*Stizostedion vitreum*). This is considerably lower than the next effect concentration of 0.93 µg L^{-1}. Above this, the progression of effect concentrations was gradual and credible. The overall GM and GSD of the nine studies recorded were 2.4 µg L^{-1} and 16, implying a 5^{th} percentile of 0.024 µg L^{-1}. Excluding the lowest value of 0.0045 µg L^{-1} gives a 5^{th} percentile of 0.38 µg L^{-1}. Both these estimates of the 5^{th} percentile are considerable lower than the lowest recorded effect concentrations. It is proposed the PNEC be set at 1 µg L^{-1} with methyl mercury as the reference compound.

The PNEC values for organic Hg were not consistently lower than those for inorganic Hg, most notably in soils. In soils, this may reflect an interaction of organic Hg with soil organic matter

Nickel

Nickel, usually present in the environment as a cation, is now regarded as a possible essential micronutrient for plants. The two lowest effect concentrations for soil microbial processes, an EC50 of 2.5 mg kg^{-1} (non-symbiotic nitrogen fixation) and a LOEC of 3 mg kg^{-1} (respiration), were indirectly cited, making evaluation difficult. The next seven lowest effect concentrations found in the literature ranged from 5 to 65 mg kg^{-1}, which is a relatively narrow range for such data and make a strong basis for setting a PNEC. The remaining effect concentrations ranged up to a LOEC of 2000 mg kg^{-1} (enumeration of fungi). The overall GM and GSD were 93 mg kg^{-1} and 6.4, implying a 5^{th} percentile of 4 mg kg^{-1}. This is consistent with the other PNEC values derived, and is proposed as the PNEC for soil microbial processes. One paper was found describing effects related to mycorrhizal species (Ajungla et al. 2003), and it dealt with measures of dehydrogenase activity in ectomycorrhizal (*Suillus luteus*, *Scleroderma aurantium*, *Cenococcum graniforme* and *Boletus* spp.) and non-mycorrhizal rhizospheric soil of pine seedlings. The EC25 was 25 mg kg^{-1}, but since this is the only information and it was based on enzyme assays instead of enumeration, it could be considered part of the general soil microbial bioassays. It is proposed that the PNEC for soil microbes be used to also represent mycorrhizae (Table 13).

There were only a few effect concentrations for soil invertebrates. The lowest value from Callahan et al. (1994) is extremely low, as was their value for Pb in soil, and so it is given little credence. The result from Boyd et al. (2001) is a LC50, a more severe endpoint than desired. In contrast, the next two lowest were LOEC and EC10, and so are probably lower than the corresponding EC25, if they were available. Similarly, the highest effect concentration was a LC50, and EC25 would be lower than this. The GM and GSD of these effect concentrations, excluding the results of Callahan et al. (1994), are 180 mg kg^{-1} and 3.5, with an implied 5^{th} percentile of 22 mg kg^{-1}, below all of the credible observed effect concentrations. The proposed PNEC is 30 mg kg^{-1}, intended as a compromise between the results of Boyd et al. (2001) and the 5^{th} percentile, recognising that this value is not based on an abundance of data.

Only five effect concentrations for freshwater plants were recorded, an EC10 of 24 µg L^{-1} (green alga *Scenedesmus subspicatus*), a LOEC of 100 µg L^{-1} (five algal species), an EC25 of 125 µg L^{-1} (*Selenastrum capricornutum*), an EC50 of 450 µg L^{-1} (duckweed), and an EC25 of 2300 µg L^{-1} (three genera of green algae). The intended effect level is EC25, between the EC10 and EC50 that were recorded. The proposed PNEC is 100 µg L^{-1}.

The lowest seven effect concentrations for aquatic invertebrates were within an order of magnitude, ranging from the lowest, an EC20 of <3.8 µg L^{-1} (cladoceran *Ceriodaphnia dubia*

Table 12. Proposed PNEC for organic mercury.

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})					
all	5		23[a]	low	Primarily methyl mercury
Aquatic (µg L^{-1})			0.01[a] 0.004[b]		
plant	0.03			low	Plant and invertebrate PNECs set from same data
invertebrates	0.03			low	
fish	1			good	
amphibians	nd				Use PNEC for fish because it has better level of certainty

[a] France (INERIS 2004)
[b] Canada (CCME 2004)

Table 13. Proposed PNEC for nickel.

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})		200[a]	50[b]		
microbial	4			good	
mycorrhizae	4			low	
invertebrates	30			low	
Aquatic (µg L^{-1})		10	25-150[b] 11[c]		
plant	100			low	
invertebrates	3			good	
fish	260			good	
amphibians	nd				Use PNEC for invertebrates because they often co-habit and there is good certainty for the PNEC

[a] Upper 95[th] percentile in Canada 63 mg kg^{-1} (unpublished)
[b] Canada (CCME 2004)
[c] Australia (ANZECC 2000)

in soft water, 50 mg $CaCO_3$ L^{-1}) and an EC25 of 3.8 µg L^{-1} (also *C. dubia*) to the seventh lowest, an EC25 of 30 µg L^{-1} (estuarine mysid shrimp *Mysidopsis bahia*). The overall GM and GSD were 70 µg L^{-1} and 7.3, implying a 5[th] percentile of 3 µg L^{-1}. This is lower than any of the recorded effect concentrations. It is important to note that EC20 were reported from Keithly et al. (2004) at four hardness levels, and the highest EC20 effect concentration was the 6.9 µg L^{-1} at a hardness of 253 mg L^{-1}. The full suite of EC20 were <3.8, 4.7, 4.0, 6.9 µg L^{-1} at hardnesses of 50, 113, 161 and 253 mg L^{-1}, supporting a PNEC nearly as low as the 5[th] percentile of recorded values. The effect concentrations for freshwater microbes ranged over 1000-fold, from an EC05 of 3 µg L^{-1} (bacteria *Pseudomonas putida*) to an EC50 of 5000 µg L^{-1} (a series of commercial microbial bioassays). This lowest effect concentration may be important to the survival of freshwater invertebrates if the organism was relevant to their foodchain, but it probably is not. The next highest effect concentration is an EC50 of 350 µg L^{-1}, which is high enough that effects on the corresponding microbes probably would not impact the invertebrates. The proposed PNEC is set at 3 µg L^{-1}, consistent with the computed 5[th] percentile and just lower than the two lowest recorded effect concentrations.

Freshwater fish are apparently less sensitive to Ni than other freshwater organisms. The three lowest effect concentrations were reasonably similar, a LC50 of 270 µg L^{-1} (fathead minnow *Pimephales promelas*), a LC50 of 350 µg L^{-1} (also *P. promelas*), and a NOEC of 466 µg L^{-1} (rainbow trout *Oncorhynchus mykiss*). All three were studies well suited for the setting of PNEC. The overall highest effect concentration

Table 14. Proposed PNEC for selenium.

Endpoint	PNEC	Upper background	Other agencies	Certainty	Comment
Soil (mg kg^{-1})		2	1-3.9[a]		
microbial	100			low	
mycorrhizae	nd				Use PNEC for plants or microbes
invertebrates	nd				Use PNEC for microbes
Aquatic (μg L^{-1})		20	1.0[a] 11[b]		
plant	2000			low	
invertebrates	2			low	Background range cited as 0.6 to 20 μg L^{-1}, PNEC will usually be above background
fish	2			good	
amphibians	nd				Use PNEC for fish

[a] Canada (CCME 2004)
[b] Australia

was a LC50 of 33 000 μg L^{-1} (striped bass *Morone saxatilis*). The overall GM and GSD were 3500 μg L^{-1} and 4.9, implying a 5th percentile of 260 μg L^{-1}. This is very consistent with the three lowest effect concentrations, and so the proposed PNEC is 260 μg L^{-1}. No data were found on which to derive a PNEC for amphibians, and so the PNEC for fish is recommended to represent amphibians.

Selenium

Selenium has two different oxidation states and the oxidation state determines its soil and plant chemistry and its potential toxicity. Only inorganic Se was considered here. Almost all of the studies dealing with Se and soil microbes were from Tabatabai and co-workers (Acosta-Martinez and Tabatabai 2001, Fu and Tabatabai 1989, Senwo and Tabatabai 1999), with a common experimental design. They added one concentration of the contaminant, and then recorded the degree of inhibition or stimulation for the specific enzyme system they were studying. This is not the preferred experimental design with a series of concentrations, but was useful for Tabatabai and co-workers because each experiment dealt with up to 25 elements and several (up to 26) soils. For Se, the effect levels reported by Tabatabai and co-workers were all inhibitory, EC33 to EC95 at concentrations of 200 or 400 mg kg^{-1} for nitrate reductase, beta-glucosaminidase, aspartase and arylamidase. The only other study recorded was Wilke (1989), whose study was unique because he used soils that had been spiked with Se (and 11 other elements) at two to three concentrations each, nine years prior to the study. He measured several endpoints, with dehydrogenase as usually the most sensitive. The NOEC he reported for Se was 5.7 mg kg^{-1}, but the tested concentrations after nine years were 1.5 (control), 5.7 and 7.4 mg kg^{-1}, not very different and not very high. It is proposed that a provisional PNEC for soil microbes be set at 100 mg kg^{-1}, recognising that this is not well supported by the literature and should be considered only for very general guidance. No data were found on which to derive a PNEC for mycorrhizae or invertebrates, and so the PNEC for soil microbes or plants is recommended to represent these organisms (Table 14).

Only the paper of Wang (1991) gives information on toxicity to aquatic plants, and this paper cites other papers by the same author. The observed effect was an EC50 at 2400 μg L^{-1}, a more severe effect level than ideal. However, because this effect concentration is three orders of magnitude larger than the PNECs for aquatic animals, it is probably irrelevant. It is proposed that the PNEC for aquatic plants be set at 2000 μg L^{-1}, recognising that this is not well founded in the literature.

The lowest effect concentration for invertebrates was a LOEC of 0.87 μg L^{-1}, but this was for a marine amphipod and was for seleno-L-methionine and seleno-DL-cystine rather than inorganic Se. The same study reported NOEC for selenite and selenate of 58 and 116 μg L^{-1}. Although Se-amino compounds may be the metabolic toxic intermediary for Se in many settings, they are not usually measured in environmental media, and so are not appropriate for PNEC. The next two lowest effect concentrations were an EC05 of 2 μg L^{-1} (protozoan *Entosiphon sulcatum*) and a NOEC of 2 μg L^{-1} (various endpoints), both of which could be considered chronic studies. Above these, the recorded effect concentrations, a LC50 of 57 μg L^{-1}, a NOEC of 58 μg L^{-1} and an EC50 of 3020 μg L^{-1}, were for acute exposures. Two effect concentrations were recorded for microbial endpoints, a LOEC of 30 μg L^{-1} (various endpoints in microcosms) and an EC25 of 6000 μg L^{-1} (Microtox).

Hamilton (2004) completed an extensive review of Se toxicity, and in it included results from a number of unpublished or difficult to obtain reports. Effect concentrations for various

Table 15. Proposed PNEC for technetium.

Endpoint	PNEC	Upper background	Certainty	Comment
Soil (mg kg^{-1})				
mycorrhizae	nd			Technetium noted to be toxic to terrestrial plants, use the PNEC for plants
Aquatic (µg L^{-1})				
all	50		low	

endpoints (not just invertebrates) as low as 2 µg L^{-1} were listed. Van Derveer and Canton (1997) proposed a sediment-based guideline, which corresponded to a water concentration as low as 3 µg L^{-1}. Roux et al. (1996) proposed a guideline concentration of 5 µg L^{-1} based on a literature review. Dobbs et al. (1996) observed a chronic ecosystem-level effect in microcosms (LOEC) at 110 µg L^{-1}. These microcosms included algae (*Chlorella vulgaris*), rotifers (*Brachionus calyciflorus*), and minnows (*Pimephales promelas*). Collectively, these are rather scant data to establish a PNEC. It is proposed that a provisional PNEC for freshwater invertebrates be set at 2 µg L^{-1}, recognising that this is not well supported by the literature and should be considered only for very general guidance.

The lowest five effect concentrations recorded for freshwater fish were fairly consistent, although they are mostly from related research groups. They are a suggested guideline of 2 µg L^{-1} (all organisms), a LOEC of 2.5 µg L^{-1} (mortality and reproduction), a LOEC of 4.8 µg L^{-1} (juvenile bluegill *Lepomis macrochirus*), a LOEC of 5 µg L^{-1} (various teratogenic effects) and an EC40 of 13 µg L^{-1} (field survey). The overall highest effect concentration was a LC50 of 19 000 µg L^{-1} (flannelmouth sucker *Catostomus latipinnis*). The overall GM and GSD were 450 µg L^{-1} and 31, implying a 5th percentile of 1.5 µg L^{-1}, quite consistent, despite the large GSD, with the guidelines set by several authors. There has been a lot of research to define a value of this PNEC, and 2 µg L^{-1} was proposed by two of the most prominent experts in the area (Hamilton and Lemly 1999). It is proposed to set the PNEC here as 2 µg L^{-1}. No data were found on which to derive a PNEC for amphibians, and so the PNEC for fish is recommended to represent amphibians.

Technetium

Since the discovery of Tc in 1937, ^{99}Tc and ^{99m}Tc have become familiar in the specialised literature. The former isotope is of concern as a waste released from nuclear installations, particularly from fuel reprocessing facilities, and the latter isotope as a waste product from medical applications. No data were found for toxicity to soil organisms other than plants. Wildung et al. (1976, 1977) suggested LOEC values for terrestrial plants at about 0.1 mg kg^{-1}. The PNEC for soil should be set based on toxicity information for terrestrial plants, not reviewed here.

Although 11 papers were found dealing with plants in solution, only three of these were truly aquatic plants (algae and duckweed). Most of the others were terrestrial plants grown in solution culture. One study used rice which is arguably an aquatic macrophyte. The lowest effect concentration was a LOEC of 100 µg L^{-1} for bush beans (*Phaseolus vulgaris*) in solution culture. The lowest effect concentration for a true aquatic photosynthetic organism was an EC60 of 310 µg L^{-1} for blue green algae (cyanobacteria) (*Anabaena cylindrica*). Across all the papers, the overall GM and GSD were 600 µg L^{-1} and 6.6, implying a 5th percentile of 27 µg L^{-1} which is substantially lower than any of the recorded effect concentrations.

Vandecasteele (1981) and Gearing et al. (1975) reported on effects on aquatic microbial processes, the later an EC25 of 50 µg L^{-1} for *Azotobacter chroococcum* in nitrogen-deficient media, and the former a LOEC of 1000 µg L^{-1} for various bacteria. Gearing et al. (1975) also included protozoans, and the corresponding LOEC was 10 000 µg L^{-1}. It is proposed the PNEC be based on the study by Vandecasteele (1981), with a value of 50 µg L^{-1} (Table 15). Obviously this is not well supported by many studies and further research would be appropriate.

INTERRELATIONSHIPS AMONG PNEC VALUES

The PNEC values derived in this report are shown in Tables 3 to 15 along with those from several national agencies. It is important to note that this report did not consider all the endpoints that may be part of the guideline development process for these national agencies. For example, the soil PNEC values here were based on organisms other than plants, whereas in many cases the national guidelines values are also intended to protect plants, livestock or human health. As a result, in cases where there appears to be little agreement, this difference in endpoints may explain the apparent discrepancy. Additionally, some national guidelines will take background concentrations into account, and this was not done here.

The relationship of the soil and aquatic PNEC values derived here is shown in Figure 1. There is a correspondence, as expected. The points representing Cd, Ni, Be, I and Sb follow a remarkable straight line in this plot, almost coincident to the best fit regression line. The point for Se is outside the range shown by the line one log unit above the best fit line, which implies the soil PNEC for Se is high relative to the aquatic

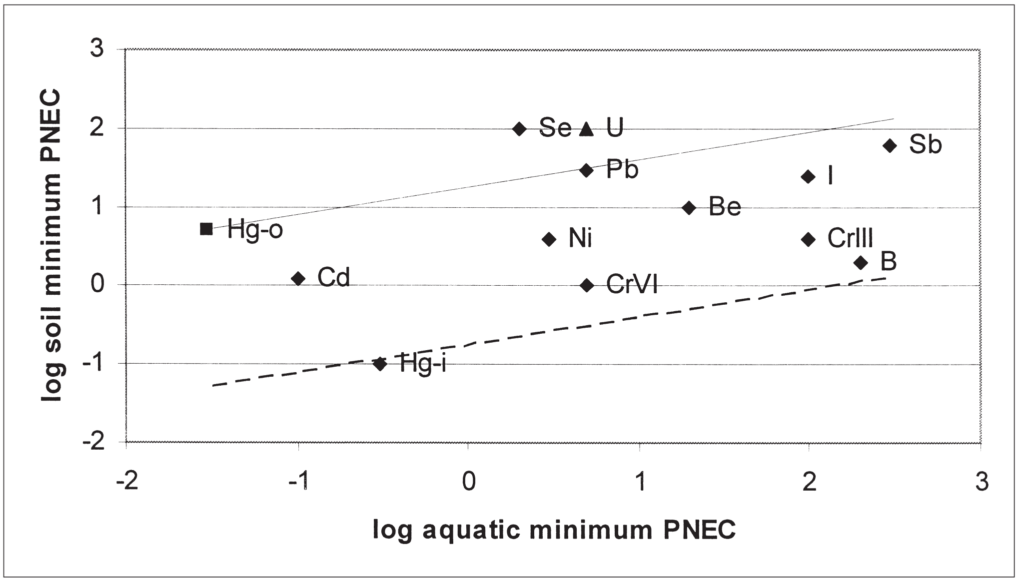

Figure 1. Comparison of soil and aquatic PNEC values derived in this project. The lines are one log unit above and below the best-fit line for these values. Hg-i refers to inorganic Hg and Hg-o to organic forms. Values for U from Sheppard et al. (2005)

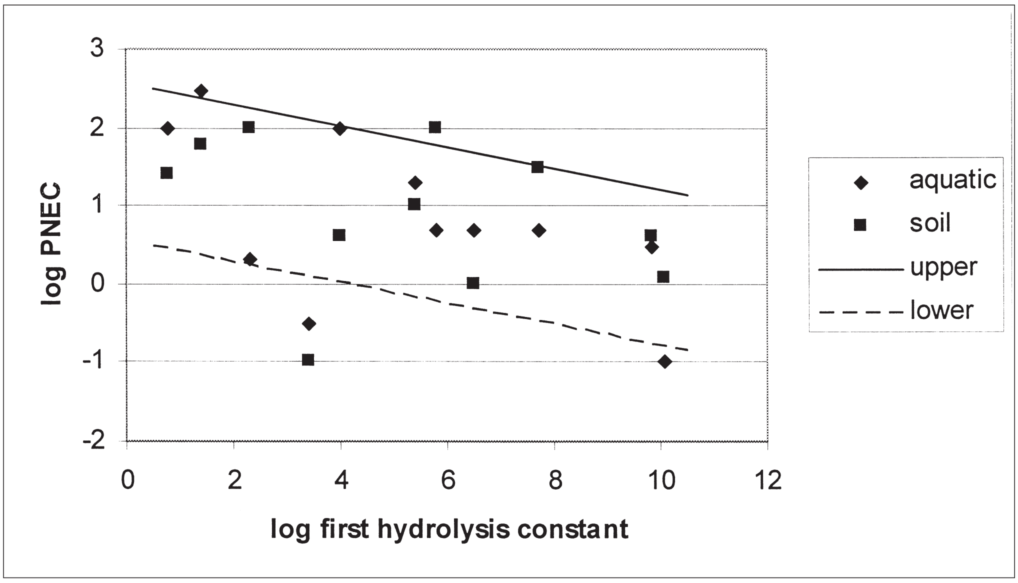

Figure 2. Plot of PNEC values versus the absolute value of the log of the first hydrolysis constant (|logK|) for each element, suggested by Newman and McCloskey (1996) as a useful abscissa. The lines are one log unit above and below the best-fit line for these values. The two points below the lower line are for inorganic Hg

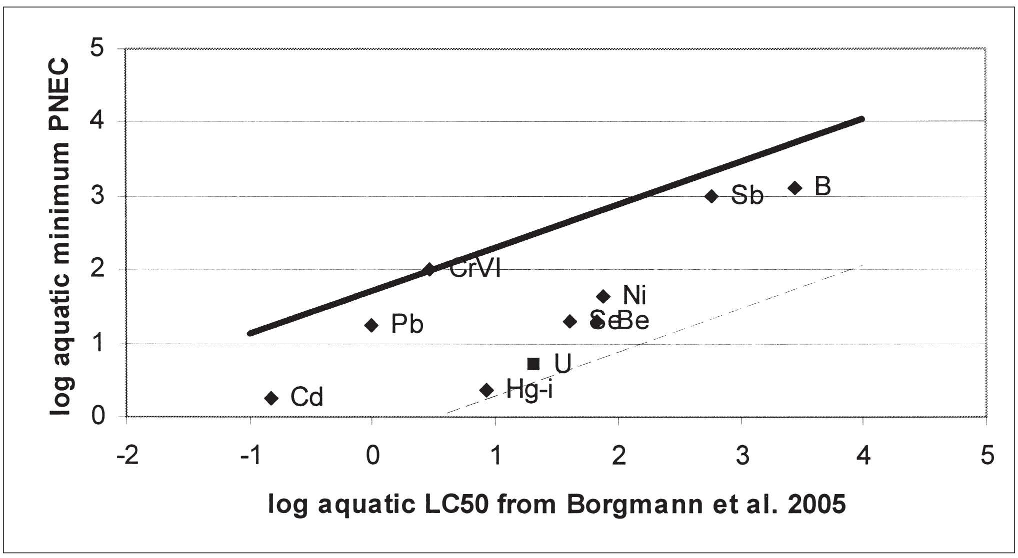

Figure 3. Comparison of aquatic PNEC values derived in this project versus LC50 values reported the multi-element study by Borgmann et al. (2005). The lines are one log unit above and below the best-fit line for these values. The value for U from Sheppard et al. (2005).

PNEC. The soil PNEC for Se is the more uncertain of the two, and this may indicate that with more data in future, the soil PNEC for Se might decrease. Similarly, the point for inorganic Hg is just lower than the line one log unit below the best fit line, and again it is the soil PNEC that is more uncertain for inorganic Hg.

Newman and McCloskey (1996) proposed the absolute value of the log of the first hydroylsis constant to be a useful abscissa for extrapolation of PNEC values from element to element. Figure 2 shows the relationship. Again, the values for inorganic Hg, both aquatic and soil, are outside the lines representing one log unit above and below the best fit line. This may indicate that the ecotoxicology of Hg is unique in some way compared to the other elements.

Although it was originally hypothesized that both these plots would represent a form of validation of the proposed PNEC values, the most they show are the expected general trends. For example, the values for Hg deviate more from the trend lines than other elements, but there seems to be no rationale that would suggest these are inappropriate PNEC values.

A similar correspondence occurred between the minimum aquatic PNEC values here and the LC50 values reported by Borgmann et al. (2005) in their multi-element toxicity tests (Figure 3). Borgmann et al. (2005) measured toxicity of 63 elements to an aquatic crustacean, an unusual study because it considered so many elements with identical test procedures. Again, the points in Figure 3 seem bounded by lines one log unit above and below the best-fit line. The interesting feature is that the points are both above and below a 1:1 line between the data sets (not shown), so that for some elements (specifically Cd, Cr(VI) and Pb), the LC50 values reported by Borgmann et al. (2005) were lower than the EC25-based PNEC values derived here. Perhaps this is because Borgmann et al. (2005) used analytical standard solutions where the solution chemistry was designed by the supplier to stabilize these elements in solution, which consequently made them bioavailable.

SUMMARY AND CONCLUSION

The nuclear industry, especially as related to storage and disposal of nuclear waste, must ensure environmental safety. However, some of the elements of potential concern are different from the elements where guidelines are normally developed. This project derived PNEC values for 11 elements, including some effects of chemical speciation among these elements. Some of the elements are widely studied and the subject of many guideline investigations, and some suffer a lack of data. The PNEC values proposed here are a self-consistent set inclusive of elements such as Sb, Be, I and Tc that are important to the nuclear industry. Without doubt, the derivation of PNEC values for these elements will evolve as more data become available.

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