NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT
BILTHOVEN, THE NETHERLANDS

Report no. 607128 001

Definition Report - Indicator Effects Toxic Substances (I_{tox})

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March, 1996

This study was performed on behalf of and commissioned by the Board of Directors of RIVM, within the framework of project 607128: "Indicator Effects Toxic Substances".

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ABSTRACT

The possibilities are studied for development of an indicator I_{tox}, which indicates the impact of toxic compounds on ecosystem health on a regional scale. I_{tox} can be used as an alternative Environmental Policy Performance Indicator for the theme of Dispersion (EPPI–D) with respect to the environmental *quality*. In future, global I_{tox}-values may also be applicable within the Global Environmental Outlooks.

The goals of this study are sixfold:

1. To study the possibilities to develop an environmental quality indicator for the theme of dispersion based on the fraction of unprotected species (F_u);
2. To develop methods for collection and assessment of input parameters for F_u-calculation of individual compounds;
3. To develop methods for aggregating F_u's of individual compounds in a single indicator;
4. To perform a test calculation of I_{tox};
5. To indicate whether development of I_{tox} is feasible and useful;
6. To design a "plan of approach" for the calculation of I_{tox}.

The proposed method of I_{tox}-calculation is based on a three-step plan, *i.e.*

1. assessment of the environmental concentrations;
2. conversion of the concentrations into impact values;
3. aggregation of the impact values into one single I_{tox}-value.

In the present report, methods were defined for all 3 steps distinguished.

Step 1: Concentration assessment for I_{tox}-calculation is based on a multimedia environmental fate box-model (Mackay-type), which is a module within the model USES (Uniform System for Evaluation of Substances) (RIVM *et al.*, 1994). From this model, field concentrations can be deduced for the separate environmental compartments, based on production volumes and several compound-specific characteristics. More accurate concentration assessments may be obtained by using (compartment-specific) emission data as input data instead of production volumes.

Step 2: The calculated field concentrations of the separate compounds are converted into a (potential) environmental effect. Starting point of this conversion is the frequency distribution function of the compound-specific log(NOEC)s. These NOECs are collected from literature on laboratory experiments with species belonging to different taxonomic groups. In Dutch chemicals management policy, the 5th percentile of a NOEC-distribution is generally defined as the Maximum Permissible Concentration (MPC) of a particular compound. Thus, based on laboratory NOECs (reproduction, growth, survival or photosynthesis) the field

concentration is determined which exceeds the NOEC of 5% of all species (the so-called Fraction unprotected (F_u)).

I_{tox}-calculation is based on the inverse method: from the NOEC-distribution function the fraction of species is determined for which the (laboratory) NOEC is exceeded at the (estimated) field concentration. F_u's may be calculated for all compounds with an estimated field concentration and frequency distribution. F_u's are regarded as a translation of the field concentration into a weighted toxicity value, *i.e.* the fraction of species for which the NOEC is exceeded. The cumulative S-shaped distribution function of NOECs is regarded as a dose-response curve of the ecosystem.

Step 3: F_u-values of all separate compounds are aggregated into a single indicator-value: I_{tox}. The proposed method of aggregation is based on similar calculation models as applied in single species combination toxicology, *i.e.* concentration addition and effect addition. In the single species case, *concentration addition* is only possible for compounds which have a similar mode of action. Application of the concentration addition model on frequency distributions of multiple species implies that not only a similar mode of action of all compounds on a single species is required, but also of one single compound on all species. In practice, this requirement is only met by the hydrophobic, inert compounds: the narcotics. Combination effects of all other compounds are calculated according to the model of *effect addition*.

Application of the effect addition model, implies that the tolerance correlation between the different compounds should be known. A tolerance correlation of $r_{AB}=1$ means that the species that is most sensitive to compound A is also most sensitive to compound B, whereas a correlation of $r_{AB}=0$ means that there is no relationship between sensitivity to compound A and B. For sake of simplicity, the idea was adopted that all combinations of compounds for which the effect addition model is applied have a tolerance correlation of $r=0$, which may be considered as a "worst case" scenario.

Two separate methods have been developed to apply the principle of F_u-aggregation:

1 - NOEC frequency distributions are calculated for each compound separately by means of the program ETX 1.3A;
- estimated field concentrations of the narcotic compounds are standardized into Hazard Units (HU) (analogous to standardization into Toxic Units in the single species case);
- for the sum of narcotic HU, $F_{u\text{NARCOTICS}}$ is determined from an "average" NOEC frequency distribution;
- $F_{u\text{NARCOTICS}}$ is aggregated with the separate F_u's of all non-narcotic compounds according to the model of effect-addition.

2. a multivariate non-linear regression is performed on the cumulative distribution of all laboratory NOECs, according to

$$F_{u_{TOTAL}} = 1 - \frac{1}{[1 + (\sum_j \frac{c_j}{a_j})^{b_{narcotics}}] \cdot \prod_i [1 + (\frac{c_i}{a_i})^{b_i}]}$$

   where F_{u}TOTAL is the aggregated F_u for all compounds, j is an individual narcotic compound with a specific concentration (c_j) and log(HC_{50}) (a_j) and a common $b_{narcotics}$ and i is an individual compound with a specific mode of action and a specific concentration (c_i), log(HC_{50}) (a_i) and b_i. In the regression analysis, F_uTOTAL is substituted by the individual, compound-specific cumulative frequency of the NOECs (*i.e.* $F_u(i)$ or $F_u(j)$), because (cumulative) frequencies of NOECs are only available for single compounds and not for combinations of compounds.

The second method is preferred, because of its simplicity, transparency and ability to start from concentrations which are not standardized into HU or log-transformed.

A test-calculation of I_{tox} has been performed with γ-hexachlorocyclohexane (lindane), pentachlorophenol, hexachlorobenzene, cadmium and copper, *i.e.* five compounds for which sufficient data are available. The I_{tox}-values for the separate environmental compartments of this test calculation correspond with the sum of PEC/PNEC-ratio which is closely related to the EPPI–D): Σ(PEC/PNEC)<1 corresponds with $I_{tox}<0.05$ and Σ(PEC/PNEC)>1 with $I_{tox}>0.05$.

Despite the fact that I_{tox}-calculation appeared to be actually possible for well-known compounds, for many compounds a lack of laboratory NOECs prevents the use of the ETX-program, or a multivariate non-linear regression. The minimum number of input data for both calculations is defined in chapter 7. In case of insufficient toxicity data, alternative methods are discussed to determine a NOEC frequency distribution. In case no toxicity data are found at all for a selected compound, it should not be taken in consideration.

Regarding I_{tox}, four fundamental choices have been made:

1. I_{tox} is based on *chronic* toxicity data. In case of insufficient chronic toxicity data, acute data may be "transformed" into chronic data by means of an assessment factor;
2. Separate indicator values are calculated for the *separate* environmental compartments;
3. I_{tox} describes the impact of toxic compounds on the environmental quality only with respect to the *ecosystem*, and not to human health;
4. I_{tox} is defined as a *regional* or *continental* indicator, based on calculated field concentrations (*e.g.* by means of USES) from production and/or emission volumes.

With respect to the last fundamental choice, it has been argued that the methods of F_u-calculation and F_u-aggregation are also applicable on a *local* scale. On a local scale, compound-specific F_u-values and their aggregated F_uTOTAL-value should be based on measured, spatially differentiated field concentrations, obtained from a monitoring network. Both individual F_u's and F_uTOTAL are suited for mapping, to indicate problem areas and historical or regional trends.

The quality of I_{tox} with respect to the characteristics of a good (environmental policy performance) indicator is shown in the scheme at the next page. This scheme results from a critical judgement by the authors. I_{tox} meets most characteristics to a satisfying level, except for its accuracy. The inaccuracy of the I_{tox}-calculation is mainly due to the uncertainty in the procedure which is caused by

1 lack of compound-specific input data to calculate a reliable field concentration;
2 the impossibility to calculate the range of uncertainty around the NOEC frequency distribution;
3 the tolerance correlation, which is set to $r=0$ in case of effect addition of compounds with specific modes of action.

It is recommended to develop additional methods to minimize and to quantify the impact of these uncertainties in future I_{tox}-calculations. Minimizing the uncertainty can be done by collecting more and better input data and by verifying the assumption that the tolerance correlation $r=0$. Quantifying the uncertainty can be done by sensitivity analysis and by development of a method to calculate the range of uncertainty around the frequency distribution function.

Based on the results of the present definition study, a plan of approach is proposed which enables calculation of an annual indicator values, *e.g.* I_{tox}-1996.

Characteristic	**I_{tox}**[1]
Scientific quality	
consensus on the goal and necessity	+
scientifically justifiable way to aggregate complex information	++
relevance of the input information	±
accuracy of the indicator value	−
reproducability of the indicator calculation	+
Dynamics	±[2]
Political relevance	
maximum level of aggregation	±[3]
possibility to define target values	+
possibility to evaluate previous and present policy	+
possibility to evaluate future policy scenarios	±
possibility to compare to other indicators	+
Recognizability and clarity	
ease of interpretation	+[4]
ease of application	+
transparency	++

1: This column indicates to what extent I_{tox} meets the characteristics of a good indicator, where −− means "I_{tox} does not meet this characteristic at all" and ++ means "I_{tox} meets this characteristic very well".

2: In principle, I_{tox} aggregates the F_u's of as many toxic compounds as possible. In practice, it is proposed to start with the list of compounds given in Appendix *IV*. In that case, I_{tox} is only expected to be sensitive to a general emission reduction of many toxic compounds together, but not to an emission reduction of a particular, single toxic compound (see 5.2.2.).

3: It was consciously decided to aggregate I_{tox} to a sub-maximum level (see 5.2.1.).

4: I_{tox} should not be interpreted as the fraction of species in the field which is unprotected, but as an indicator-value (0-1) for the potential risk of toxic compounds for ecosystems, which is based on laboratory toxicity data (see 5.2.4.). Risk assessment is based on F_u's in conformity with the starting points of the present chemicals management policy in The Netherlands. The actual meaning of exceeding the MPC (*i.e.* $F_u \geq 0.05$) in terms of effects on ecosystems is studied within the framework of RIVM-project 607506 (Effect Assessment Milieu II) and is not under discussion in the present definition report.

SAMENVATTING

De mogelijkheden zijn onderzocht om een indikator (I_{tox}) te ontwikkelen, die in staat is om op regionale schaal het effekt van toxische stoffen op de gezondheid van ecosystemen aan te geven. Met betrekking tot de milieukwaliteit, kan I_{tox} worden toegepast als een alternatief voor de huidige Milieubeleidsindikator-Verspreiding (MBI-V). In de toekomst kunnen mondiale I_{tox}-berekeningen eveneens worden toegepast binnen de Wereldmilieuverkenningen.

Zes verschillende doelen zijn geformuleerd voor deze definitiestudie:
1 Het onderzoeken van de mogelijkheden om een milieu*kwaliteits*indikator te ontwikkelen voor het thema Verspreiding gebaseerd op de fraktie onbeschermde soorten (F_u);
2 Het ontwikkelen van methoden om invoer parameters te verzamelen of te schatten;
3 Het ontwikkelen van methoden om F_u-waarden van afzonderlijke stoffen te aggregeren tot één enkele indikator-waarde;
4 Het uitvoeren van een proefberekening van I_{tox};
5 Het aangeven van de haalbaarheid en het nut van I_{tox};
6 Het opstellen van een plan van aanpak voor de I_{tox}-berekening.

De voorgestelde methode voor I_{tox}-berekening bestaat uit een drie-stappenplan:
1 schatting van de milieuconcentraties;
2 omrekening van de concentraties in effektwaarden;
3 aggregeren van deze effekt waarden in één I_{tox}-waarde.

Stap 1: Concentratiebepaling voor de I_{tox}-berekening is gebaseerd op een Mackay-type boxmodel, dat een module vormt binnen UBS (Uniform Beoordelingssysteem van Stoffen) (RIVM *et al.*, 1994). Uit dit model kunnen veldconcentraties worden afgeleid voor de afzonderlijke milieucompartimenten, gebaseerd op produktie volumes en sommige stof-specifieke eigenschappen. Wanneer (compartiment-specifieke) emissiegegevens worden ingevuld in plaats van produktiecijfers, kunnen concentraties nauwkeuriger worden geschat.

Stap 2: De berekende veldconcentraties van de verschillende stoffen worden omgerekend naar een (mogelijk) milieu-effekt. Hierbij wordt uitgegaan van de frequentieverdelingsfunktie van de stofspecifieke log(NOEC). Deze NOECs zijn verkregen uit laboratorium-experimenten, beschreven in de openbare literatuur, met soorten uit verschillende taxa. In het Nederlandse stoffenbeleid wordt het 5e percentiel van de NOEC-verdelingsfunktie in het algemeen beschouwd als het Maximaal Toelaatbaar Risico (MTR) van een bepaalde stof. Met andere woorden, op basis van laboratorium NOECs (reproduktie, groei, overleving, fotosynthese) wordt de veld concentratie bepaald waarbij de NOEC wordt overschreden voor 5% van alle soorten (de zogenaamde Fraktie onbeschermde soorten (F_u)).

De I_{tox}-berekening maakt gebruik van de omgekeerde methode: uit de NOEC-verdelingsfunktie wordt de Fraktie onbeschermde soorten (F_u) bepaald, waarvoor de geschatte veldconcentratie de laboratorium-NOEC overschrijdt. De S-vormige cumulatieve verdelingsfunktie wordt beschouwd als een dosis-effekt curve van het ecosysteem. F_u kan worden bepaald voor alle stoffen waarvoor een veldconcentratie én een NOEC frequentieverdeling geschat kan worden. F_u's mogen worden beschouwd als een vertaalslag van de veldconcentratie in een gewogen effektwaarde.

Stap 3: F_u-waarden van de afzonderlijk stoffen worden geaggregeerd naar één enkele indikatorwaarde: I_{tox}. De voorgestelde methode van aggregeren is gebaseerd op dezelfde rekenmodellen die worden toegepast in de "single species" combinatie-toxicologie: concentratie-additie en effekt-additie. In het "single species"-geval is *concentratie-additie* alleen mogelijk voor stoffen met hetzelfde werkingsmechanisme. Toepassing van het concentratie-additie-model op de frequentieverdelingsfunkties van meerdere soorten schept de voorwaarde dat niet alleen hetzelfde werkingsmechanisme geldt voor alle stoffen op dezelfde soort, maar ook van één enkele stof op alle soorten. In de praktijk voldoen alleen de hydrofobe, inerte stoffen aan deze voorwaarde: de narcotica. Combinatie-effekten van alle andere stoffen worden berekend volgens het model van *effekt-additie*.

Gebruik maken van het effekt-additie-model betekent dat de tolerantie correlatie bekend moet zijn. Een tolerantie correlatie van $r_{AB}=1$ betekent dat dezelfde soort die het meest gevoelig is voor stof A ook het meest gevoelig is voor stof B, terwijl $r_{AB}=0$ betekent dat er geen relatie bestaat tussen de gevoeligheid van een soort voor stof A en B. Om het rekenwerk te vereenvoudigen is besloten de tolerantie correlatie tussen all combinaties van stoffen waarvoor het effekt-additie-model wordt toegepast te definiëren als $r=0$. Dit mag als een "worst case" scenario worden beschouwd.

Twee verschillende methodes zijn ontwikkeld om het F_u-principe toe te passen:

1 - berekenen van NOEC frequentieverdelingen voor elke stof afzonderlijk middels het programma ETX 1.3A;
  - standaardiseren van de geschatte veldconcentraties in Hazard Units (HU) (vergelijkbaar met standaardiseren in Toxic Units in het "single species"-geval.
  - berekenen van de $F_{u\text{NARCOTICA}}$ voor de gesommeerde concentratie van narcotische stoffen (in termen van HU) met behulp van de "gemiddelde" NOEC frequentieverdeling.
  - aggregeren van de $F_{u\text{NARCOTICA}}$ met de afzonderlijke F_u's van de niet-narcotiserende stoffen volgens het model van effekt-additie.

2 uitvoeren van een multivariate non-lineaire regressie op de cumulatieve verdeling van alle NOECs vastgesteld in toxiciteitsexperimenten in het laboratorium, volgens

$$F_{u_{TOTAAL}} = 1 - \frac{1}{[1 + (\sum_j \frac{c_j}{a_j})^{b_{narcotica}}] \cdot \prod_i [1 + (\frac{c_i}{a_i})^{b_i}]}$$

waarin F_{u}TOTAAL is de geaggregeerde F_u voor alle stoffen, j is een afzonderlijke narcotiserende stof met zijn eigen specifieke concentratie (c_j) en log(HC_{50}) (a_j) en een gemeenschappelijke b_j met alle overige narcotiserende stoffen, en i is een afzonderlijke stof met een specifiek werkingsmechanisme en een stof-specifieke concentratie (c_i) en log(HC_{50}) (a_i) en b_i. In de regressie-analyse kan F_{u}TOTAAL gesubstitueerd worden door de afzonderlijke, stof-specifieke cumulatieve frequentie van NOECs ($F_u(i)$ of $F_u(j)$), aangezien er geen NOECs beschikbaar zijn voor combinaties van stoffen, maar alleen voor afzonderlijke stoffen.

De tweede methode wordt geprefereerd vanwege zijn simpelheid, zijn "doorzichtigheid" en zijn mogelijkheid om met concentraties te werken die niet zijn gestandaardiseerd in HU of log-getransformeerd.

Een proefberekening van I_{tox} is uitgevoerd voor de stoffen γ-hexachlorocyclohexaan (lindaan), pentachlorophenol, hexachloorbenzeen, cadmium and koper. Voor al deze 5 stoffen zijn voldoende invoer-gegevens beschikbaar om veldconcentraties en NOEC frequentieverdelingen te berekenen. Per milieucompartiment komt de uitkomst van de I_{tox}-berekening overeen met de uitkomst van de PEC/PNEC-ratio, die nauw verwant is aan de huidige Milieubeleidsindikator voor het thema Verspreiding: Σ(PEC/PNEC) < 1 komt overeen met $I_{tox} < 0.05$ en Σ(PEC/PNEC) > 1 met $I_{tox} > 0.05$.

Ondanks het feit dat I_{tox}-berekening haalbaar bleek voor bekende stoffen, is er een gebrek aan laboratorium NOECs voor vele stoffen, waardoor geen gebruik kan worden gemaakt van het ETX-programma, of van multivariate, non-lineaire regressie. Het minimum aantal van invoer-gegevens voor beide methoden is gedefinieerd in Hoofdstuk 7. In het geval van onvoldoende toxiciteitsgegevens, worden alternatieve methoden besproken om een NOEC frequentieverdeling te kunnen schatten. Wanneer voor een bepaalde stof geen enkel toxiciteitsgegeven bekend is, kan deze stof niet worden meegenomen in de I_{tox}-berekening.

Vier principiële keuzes zijn gemaakt met betrekking tot I_{tox}:

1 I_{tox} is gebaseerd op *chronische* toxiciteitsgegevens. In geval van onvoldoende chronische toxiciteitsgegevens kunnen acute gegevens worden "getransformeerd" in chronische gegevens met behulp van een schattingsfaktor;

2 Afzonderlijke indikator-waarden worden berekend voor de *afzonderlijke* milieucompartimenten;

3 I_{tox} geeft een indruk van het effekt van toxische stoffen op milieukwaliteit alleen ten

aanzien van het *ecosysteem*, en niet ten aanzien van de volksgezondheid;
4 I_{tox} is gedefinieerd als een *regionale* of *continentale* indikator, gebaseerd op berekende veldconcentraties (bijvoorbeeld middels UBS) op basis van produktie- en/of emissievolumes.

Ondanks deze laatste principiële keuze, is de methode van F_u-berekening en F_u-aggregatie ook toepasbaar op *lokale* schaal. Op lokale schaal moeten stof-specifieke F_u-waarden en de geaggregeerde $F_{u\text{TOTAAL}}$-waarde gebaseerd zijn op gemeten, ruimtelijk-gedifferentieerde veldconcentraties, die bepaald zijn binnen een monitoring-netwerk. Zowel individuele F_u's als $F_{u\text{TOTAAL}}$'s kunnen worden gekarteerd om probleemgebieden aan te geven, of historische of regionale tendensen.

De kwaliteit van I_{tox} met betrekking tot de eigenschappen van een goede (milieubeleids-) indikator is weergegeven in het schema op de volgende pagina. Dit schema komt voort uit een kritische beoordeling van I_{tox} door de auteurs. I_{tox} voldoet aan de meeste van deze eigenschappen, met uitzondering van de nauwkeurigheid. De onnauwkeurigheid van de I_{tox}-berekening is voornamelijk te wijten aan onzekerheid die wordt veroorzaakt door:

1 een gebrek aan invoergegevens om een betrouwbare veldconcentratie te berekenen;
2 de afwezigheid van een methode om het onbetrouwbaarheidsgebied rond de NOEC frequentieverdeling te berekenen;
3 de tolerantie-correlatie, die gelijkgesteld is aan $r=0$ in geval van effekt-additie van stoffen met een specifiek werkingsmechanisme.

Het wordt aanbevolen om aanvullende methoden te ontwikkelen om de invloed van de onnauwkeurigheid op in toekomstige I_{tox}-berekeningen te minimaliseren en te kwantificeren. Minimalisering van de onnauwkeurigheid kan worden bereikt door meer en beter invoergegevens te verzamelen en door de aanname dat de tolerantie correlatie $r=0$ te verifiëren. Kwantificeren van de onnauwkeurigheid kan worden bereikt door gevoeligheidsanalyse en door de ontwikkeling van een methode waarmee het onbetrouwbaarheidsgebied rond de NOEC frequentieverdeling kan worden berekend.

Op basis van de resultaten van deze definitiestudie word een plan van aanpak voorgesteld waarmee jaarlijkse indikatorwaarden berekend kunnen worden, zoals I_{tox}-1996.

Eigenschap	I_{tox}[1]
Wetenschappelijke kwaliteit	
consensus over doel en noodzaak	+
wetenschappelijk verantwoorde manier om complexe informatie te aggregeren	++
relevantie van de invoergegevens	±
nauwkeurigheid van de indikator-waarde	−
herhaalbaarheid van de indikator-berekening	+
Dynamiek	±[2]
Politieke relevantie	
maximaal niveau van aggregeren	±[3]
mogelijkheid om doel-waarden te definiëren	+
mogelijkheid om gevoerde en huidige beleid te evalueren	+
mogelijkheid om toekomstige beleidsscenario's te evalueren	±
mogelijkheid om met andere indikatoren te vergelijken	+
Herkenbaarheid en doorzichtigheid	
gemakkelijke interpretatie	+[4]
gebruiksvriendelijkheid	+
doorzichtigheid	++

1: Deze kolom geeft aan in hoeverre I_{tox} voldoet aan de eigenschappen van een goede indikator, waarbij −− betekent "I_{tox} voldoet helemaal niet aan deze eigenschap" en ++ betekent "I_{tox} voldoet helemaal aan deze eigenschap";

2: In principe worden F_u-waarden van zoveel mogelijk stoffen geaggregeerd in I_{tox}. In de praktijk wordt voorgesteld om te beginnen met de lijst van stoffen uit Appendix *IV*. In dat geval wordt I_{tox} verondersteld gevoelig te zijn voor een algemene emissie reductie van veel toxische stoffen tegelijkertijd, maar niet voor een emissie reductie van één bepaalde toxische stof.

3: I_{tox} is bewust slechts geaggregeerd tot een sub-maximaal niveau (zie 5.2.1.).

4: I_{tox} moet niet worden beschouwd als de fraktie soorten die onbeschermd is in het veld, maar als een indikatorwaarde (0-1) voor het potentiële risico van toxische stoffen voor ecosystemen, gebaseerd op toxiciteitsgegevens uit laboratoriumexperimenten (zie 5.2.4.). Risicoschatting is gebaseerd op F_u's conform de uitgangspunten van het huidige Nederlandse Stoffenbeleid. De werkelijke betekenis van normoverschrijding (m.a.w. $F_u \geq 0.05$) in termen van effekten op ecosystemen wordt bestudeerd in RIVM-projekt 607506 (Effect Assessment Milieu II) en is in dit rapport buiten diskussie gelaten.

1. INTRODUCTION

1.1. Environmental Policy Performance Indicators

The current Dutch environmental policy focusses both on the environmental issues (called themes) and on the causes of the problems which are divided into sectors (called target groups). One of the environmental issues defined in the Dutch National Environmental Policy Plan (NEPP, 1989) is the theme of dispersion. This theme concerns the pollution with and the adverse environmental effects of environmentally hazardous substances and of genetically modified organisms on man, flora and fauna.

In order to enable assessment of the impact of policy measures on the state of affairs of the environmental themes, separate Environmental Policy Performance Indicators (EPPI) were developed per theme (Adriaanse, 1993). Each indicator contains theme-specific condensed information on the pressure (*i.e.* the stress factor which causes the environmental problem) and the possible effects of this pressure in a certain area (*e.g.* The Netherlands). For the theme of dispersion this implies that the indicator should contain information on either emissions (pressure) or concentrations or environmental effects which proceed from these emissions[1].

At present, the Environmental Policy Performance Indicator for the theme of Dispersion (EPPI–D) is defined as (Adriaanse, 1993):

$$EPPI\text{-}D = \sum_i \left(\frac{E_i}{MPC_i} \cdot T_i\right).$$

This indicator is based on the sum of all emissions (E_i in [kg/s]), which are weighted for decay/decomposition by means of a correction factor (T_i in [s]) and for toxicity by the maximum permissible concentration (MPC_i in [kg/m^3]). The equation above results in an outcome with dimension [m^3], and should be interpreted as the environmental volume which is filled up to the maximum permissible concentration by toxic compounds.

1.1.1. Need for further definition and explanation of the EPPI–D

The outcome of the present EPPI–D calculation is easy to understand in terms of environmental volume. Its interpretation becomes even more perceptible, when EPPI–D is

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[1] In the present report, the possibilities are described for development of a (policy) indicator for the theme of dispersion. The relative importance of this theme compared to the other themes (*e.g.* change of climate, depletion of ozone layer, acidification) is not under discussion.

is divided by the total environmental volume (V) and thus made dimensionless:

$$\frac{EPPI\text{–}D}{V} = \frac{1}{V}\cdot\sum_i \frac{E_i\cdot T_i}{MPC_i} = Q$$

T_i has dimension time [s] and its inverse $k_i = 1/T_i$ [s^{-1}] may be regarded as a first order disappearance rate for compound i. Substituting T_i by $1/k_i$ results in the following equation

$$Q = \frac{1}{V}\cdot\sum_i \frac{E_i\cdot T_i}{MPC_i} = \frac{1}{V}\cdot\sum_i \frac{E_i/k_i}{MPC_i} = \sum_i \frac{E_i/(k_i\cdot V)}{MPC_i}$$

The nominator of this equation can be regarded as the Predicted Environmental Concentration (PEC):

$$PEC_i = \frac{E_i}{k_i\cdot V}$$

whereas the denominator MPC is regarded as the concentration which poses a maximum permissible risk, and which previously was believed to cause no effects to the ecosystem structure (the so-called predicted no-effect concentration (PNEC)). The indicator Q is dimensionless and should be interpreted as a fraction of the total environmental volume which is filled up to the PNEC. Dividing the original EPPI–D by V

1. implies that in fact a volume goal is set for EPPI–D, *i.e.* the total environmental volume (per compartment) and
2. comes down to calculation of the PEC/PNEC ratio.

Q has a target value of 0 and a maximum permissible value of 1.

Despite the good characteristics of EPPI–D described above, further definition of the indicator is necessary. Furthermore, the equation implies certain assumptions which give rise to objections when stated explicitly. This is illustrated below:

- No goal is set for the (overall) environmental volume which is allowed to be filled up by the annual emissions. For toxic compounds, environmental policy objectives are only set per compound and per compartment (*i.e.* maximum permissible concentrations). No target volume is set for separate compounds or all compounds together.
- The parameters E_i, T_i (k_i) and MPC_i are defined insufficiently, *e.g.* for which environmental compartment (soil, sediment, water, air) and on what scale (regional or local)? Furthermore, it is not clear which disappearance processes are represented by k_i.
- After proper definition of the parameters E_i, T_i (k_i) and MPC_i, for most compounds insufficient data are available for EPPI–D-calculation. Therefore, clear procedures should be defined to make an assessment of the values of the parameter.
- Within the calculation of EPPI–D, volumes of different environmental compartments

(which are filled up to their specific MPC) are added to an overall environmental volume without attributing a weighing factor to these compartments. This implies that using 1 m^3 of air equals the use of 1 m^3 of soil.

- Summation of the PEC/PNEC ratios implies that the overall toxic effect of all compounds together is assessed by summation of all separate concentrations, which are weighted for their individual toxicity by means of the MPC. In other words, concentration-addition is assumed for all compounds. However, this assumption is only valid when compounds show similar action. If this is not the case, different methods need to be developed to aggregate effects of toxic compounds.

1.1.2. Pressure indicator *vs* Quality indicator

With regard to the first objection, it is difficult to set an intuitive goal for the environmental volume which may be filled up to the limit. On the other hand, policy objectives have been set in the Netherlands for some compounds with respect to (1) emission and/or (2) maximum permissible concentrations in the environment. Therefore, it is proposed to develop two separate indicators with respect to dispersion:

1 a *Pressure indicator* which indicates the pressure on the ecosystem by emission of toxic compounds and takes account of the remarks listed in above;
2 a *Quality indicator* which indicates the quality of the ecosystem in terms of the concentration of toxic compounds.

(1) Pressure indicator

Emission goals were defined for several compounds regarded as "attention substances" (Janus *et al.*, 1994). To indicate the distance of the present situation to the goals defined by environmental policy plans, EPPI–D may be redefined as

$$EPPI\text{–}D = \sum_i \frac{E_i - E_{goal}}{E_{goal}}.$$

This indicator has a target value of 0. Major drawback of this indicator is that no account is taken of the spatial scale at which the emissions exceed the emission goals. In other words, no difference is made between local and regional emissions exceeding the goals and both kind of emissions are added without any weighing. At present, a project group of experts is working to resolve this problem by attributing weighing factors to local and regional emissions (Bodar *et al.*, 1995).

(2) Quality indicator

Within Dutch chemicals management policy, maximum permissible concentrations (MPCs) per compartment have been defined for the separate compounds. As stated above, MPC is regarded as PNEC. The MPC is based on the distribution of the sensitivity (determined as No Observed Effect Concentrations (NOECs) in laboratory experiments) of as many different species as possible. The MPC is defined as the concentration at which the NOEC is exceeded for 5% of all species, *i.e.* laboratory experiments indicate that at MPC, 5% of all species may be negatively affected by the toxic compound with regards to vital processes which are essential for survival of the populations (reproduction, growth, survival or photosynthesis). Thus, calculating Q comes down to calculating the ratio between the PEC and a fixed concentration at which 5% of all species tested in the laboratory are negatively affected.

However, in order to indicate the quality of the environment, it is more powerful to calculate directly the fraction of test species for which the laboratory NOEC is exceeded under field conditions (the so-called Fraction unprotected (F_u)), rather than to compare the field concentration to the concentration at which 5% of the species is possibly affected. Aggregating the compound-specific F_u's yields a $F_{u\mathrm{TOTAL}}$ per compartment, which may be regarded as a quality indicator of the environment, with regard to toxic compounds. From now on, this indicator will be called "Indicator toxic compounds" (I_{tox}). I_{tox} is defined as the total fraction of species tested under laboratory conditions for which the NOEC is exceeded as a result of the simultaneous presence of many chemicals under field conditions:

$$I_{tox} = F_{uTOTAL} = \underset{i}{\Psi} F_{u_i}$$

where Ψ is the method of aggregating the separate F_u's, which should be defined in this report[2]. For I_{tox}, no maximum permissible value is derived yet, although the F_u's for individual compounds have a maximum permissible value of 5%.

1.2. Problem definition

The main question of the present study is whether it is possible and relevant to develop an EPPI-like indicator (I_{tox}) for the theme of dispersion which is merely based on the quality of the environment. This study mainly investigates the possibility and feasibility to collect information on the presence of toxic compounds and their possible effects on natural populations in a certain area and to aggregate this information into a single indicator-value.

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[2] I_{tox} is based on the fraction of species tested in laboratory experiments for which the NOEC is exceeded under field conditions. This is in conformation with the present chemicals management policy in The Netherlands which also starts from the idea that ecosystem protection can be achieved based on limiting the fraction of species which may be exposed to toxicant levels above their NOEC. The actual meaning of exceeding the NOEC for a (limited) fraction of species in terms of effects on ecosystems is studied within the framework of RIVM-project 607506 (Effect Assessment Milieu II) and is not under discussion in the present definition report.

Starting point of the method of aggregation is that I_{tox} expresses the magnitude of the risk which chemicals in the environment pose to the natural inhabitants (preferably in terms of F_u).

One of the main problems is the availability of input data. For many compounds, insufficient toxicity data are available to calculate a sensitivity distribution function (Van de Plassche, 1994) whereas measured field concentrations are also scarce and only available for "attention substances". Therefore, an inventory is needed of the input parameters and their availability for the F_u-calculation. In case of a lack of input parameters, assumptions regarding these parameters are necessary. These assumptions should be founded on a scientific basis and be described in standardized procedures.

Ideally, individual F_u-values can be calculated per compound per compartment. No method has been developed yet to aggregate these separate F_u's to a $F_{u\text{TOTAL}}$ per compartment. Such method is described in this report, together with the assumptions on which this method is based.

Finally, fundamental choices have to be made concerning the aggregation of F_u's:

1 Time: should the sensitivity distribution function be based on acute or chronic laboratory experiments?
2 Compartments: is it useful and scientifically sound to aggregate the I_{tox}'s per compartment to an overall I_{tox}? Or is it sufficient to develop separate I_{tox}'s per compartment?
3 Eco *vs* human: is it worth developing separate environmental quality indicators for humans and for ecosystems, or should these two be integrated in a single indicator?
4 Scale: should I_{tox} be calculated on a national (regional) or local scale?

1.3. Goals of this definition study

The goals of this study are sixfold:

1 To study the possibilities to develop an environmental quality indicator for the theme of dispersion based on the fraction of unprotected species (F_u);
2 To develop methods for collection and assessment of input parameters for F_u-calculation of individual compounds;
3 To develop methods for aggregating F_u's of individual compounds in a single indicator;
4 To perform a test calculation of I_{tox};
5 To indicate whether development of I_{tox} is feasible and useful;
6 To design a "plan of approach" for the calculation of I_{tox}.

1.4. Framework of this definition study

1.4.1. Applicability

This definition study has been performed on behalf of the Board of Directors of RIVM. Calculation of I_{tox}-1996 is planned within the framework of the project Mapping Ecotoxicological Effects of Substances (MEES) which will start in 1996 on behalf of the Directorate-General for Environmental Protection. The calculated I_{tox}-1996 will probably also be reported in the Environmental Outlook 1997.

Within the framework of MEES, not only I_{tox} will be calculated, but local F_u-values will also be mapped, to enable distinction between areas with more and less toxic pollution. F_u-maps will be developed for separate compounds, but also for specific combinations of compounds and all compounds together. The methods for F_u-calculation and F_u-aggregation proposed in the present report is also applicable within this part of the MEES-project.

Finally, I_{tox} may be applicable within the framework of the Global Environmental Outlooks (GEO) in which environmental problems are studied on a global scale. Within GEO, a mathematical model called TARGETS (Rotmans *et al.*, 1994) is used to describe long term trends per compartment. TARGETS enables analysis of differences between separate years, recognition of causes for the course of a certain trend and prediction of the effectiveness of different measures. In order to keep a clear structure of the model, all relevant processes for the state of the environment on a global scale have to be simplified and aggregated. For each environmental problem (*e.g.* acidification, greenhouse effect), relevant information from the model (usually a measurable parameter) is comprised within a hierarchical framework ("tree") into an aggregated indicator. Next, the aggregated indicators per theme are further aggregated to an environmental top index by means of weighing factors, which are attributed by expert judgement, based on the potential impact of that particular environmental problem.

Separate environmental top indices were derived with respect to (*i*) the pressure on, (*ii*) the state of and (*iii*) the impact on the global environment. Up till present, toxification of the environment has not been processed in these indices, because no separate indicators have been developed for this environmental problem. The pressure and quality indicators for the theme of dispersion (see 1.1.2) may also be applicable as indicators for toxification within the pressure and state "tree" of the TARGETS model.

1.4.2. Tuning

A group of experts (mainly from RIVM; see Appendix *I*) was interviewed on:

- the necessity of an environmental quality indicator with respect to toxic compounds;

- the goal of this indicator;
- the possibility to base this indicator on F_u;
- possible methods to aggregate F_u's of the individual compounds.

Based on these interviews a paper was written (Hamers, 1995a) which was discussed in a workshop held at RIVM on the 19th of September, 1995 (Hamers, 1995b).

1.5. Outline of this report

The outline of this report is as follows:
In chapter 2 functions, types and characteristics of indicators are described. In chapter 3, methods for F_u-assessment and F_u-aggregation have been worked out which are applied in the test calculation described in chapter 4. The methods and results of this calculation are discussed in chapter 5, which leads to the conclusions of chapter 6. In chapter 7 a plan of approach is presented to calculate I_{tox}. Finally, this report is closed by a list of references and six Appendices.

2. POLICY INDICATORS

2.1. Functions of policy indicators

In general, policy indicators have 3 functions, *i.e. simplification*, *quantification* and *communication*. Indicators simplify large amounts of information in order to quantify a complex phenomenon by which means communication on that phenomenon is started or improved. Furthermore, target values can be set for future years and trends from past years can be visualized based on the outcome of an indicator (Adriaanse, 1993).

In particular, I_{tox} should be able to express the magnitude (state of affairs) of the problem of dispersion with respect to the policy targets defined for this theme and in proportion to the other themes distinguished in Dutch environmental policy.

2.2. Types of policy indicators

A first classification of policy indicators is based on which phase of policy making the indicator is aimed at. Indicators can be used:

- to evaluate different alternative policy measures and to predict future developments. Such indicator is called a *prospective* indicator;
- to evaluate the chosen policy, to represent trends and to indicate whether the indicator shifts into the direction of a defined target value. Such indicator is called a *retrospective* indicator.

A second classification of policy indicators is based on which step of the so-called PSR-chain is represented by the indicator. This chain consists of three major steps, *i.e.* the pressure (P) or stress on the environment, the state (S) of the environment and the response (R) by the human society to decrease the pressure or to improve the state of the environment (OECD, 1991). For EPPI's, this PSR-model has been worked out in Figure 2.1 (Adriaanse, 1993).

Based on this classification, indicators may be developed for each separate step. *E.g.*, for the theme of dispersion the following indicators can be distinguished:

- *Pressure*-indicators, representing the causes (stress factors) of the environmental problems, such as emission, deposition and use of energy and resources;
- *State*-indicators, representing the environmental quality of a certain area in terms of concentrations of pollutants and their connected (expected) effects;
- *Response*-indicators, representing the efforts and measurements taken to prevent and decrease toxic pressure and/or to improve the state of the environment.

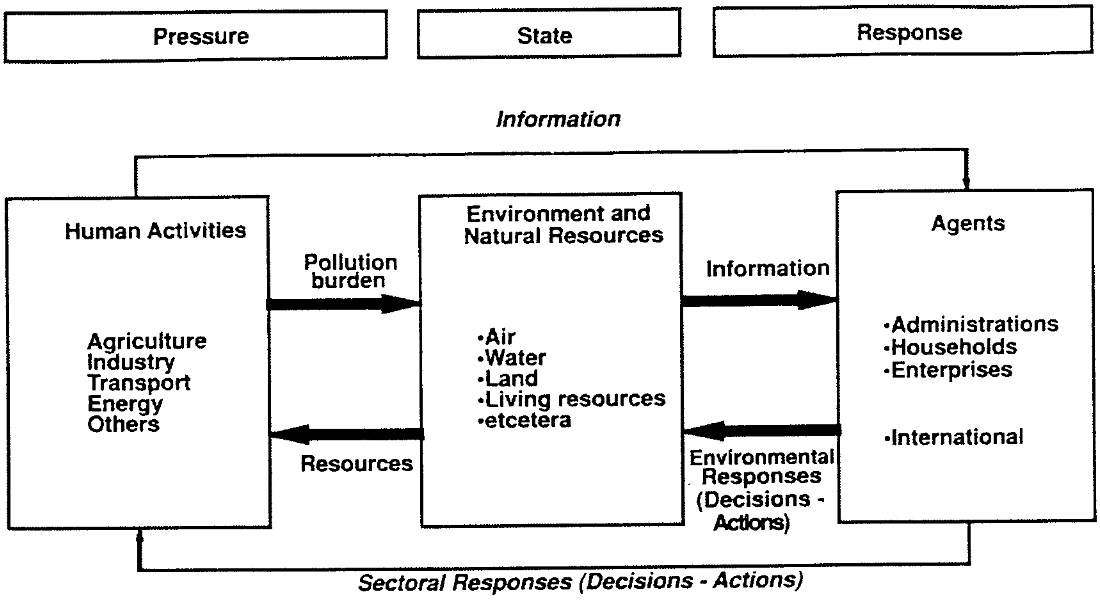

Figure 2.1: The Pressure-State-Response model within a conceptual framework for the Environmental Performance Indicators (from: Adriaanse, 1993).

2.3. Characteristics of policy indicators

Four characteristics determine the quality of a policy indicator (Adriaanse, 1993; expert interviews, present study):

1 *Scientific quality*, which is realized by
  - consensus on the goal and necessity;
  - a scientifically justifiable way of aggregating complex information;
  - aggregating relevant input information to indicate the environmental quality;
  - high accuracy of the indicator value;
  - reproducability of the indicator calculation.

2 *Dynamics*, which is demanded since (long-term) trends should be visualized by means of the indicator. This is a very important characteristic for a good indicator.

3 *Political relevance*, which is realized by the possibility
  - to achieve a maximum level of aggregation;
  - to define target values;
  - to evaluate previous and present policy;
  - to evaluate future policy scenarios;
  - to compare the indicator value to indicators for different environmental themes, which enables priority setting in policy making.

4 *Recognizability and clarity*, which are probably the most important characteristics. An indicator needs to be

- expressive, *i.e.* the outcome of the indicator-calculation is easy to interpret for users of the indicator (both politicians and citizens);
- an accessible instrument, which is easy to apply. In other words, input information should be easy available and calculation should not be too difficult;
- "transparent", despite the complex information which has been aggregated for many compounds into the single indicator value. Dynamics of the basic processes should be reflected by the dynamics of the indicator value.

Based on the methods developed and the outcome of the test calculation, the characteristics of a good indicator will be discussed with respect to I_{tox} in 5.2.

3. PRINCIPLES OF F_u-ASSESSMENT AND F_u-AGGREGATION

As proposed in chapter 1, I_{tox} will be based on the fraction of all species for which the NOEC is exceeded in the field situation (F_u). Therefore, in 3.1. a method is proposed for F_u-assessment for single compounds and in 3.2. methods are proposed to aggregate F_u's for different substances into a single I_{tox}-value.

3.1. Principles of F_u-assessment

For F_u-assessment of a single compound, it is fundamental to know (*i*) the actual concentration in the field and (*ii*) the sensitivity distribution function of species tested under laboratory conditions. Assessment of both aspects and the actual F_u-assessment itself is described in separate paragraphs.

3.1.1. Concentration assessment

Generally, I_{tox} should be able to indicate the toxic pressure of all compounds present in the environment. Since the toxicity of a compound is dose-dependent, availability of environmental concentrations is a critical condition for F_u-assessment. Environmental concentrations may either be calculated or measured.

For F_u-assessment, *measured* field concentrations can be used as input data, which are usually determined within the framework of a monitoring program. Within such programs, concentrations are usually measured for a very limited group of substances. Despite the fact that this group of compounds is considered to be responsible for a considerable part of the toxic stress in a certain area, it is regarded as a disadvantage that I_{tox} will be based on a small number of compounds. Furthermore, I_{tox} is meant to be a national indicator for toxic stress on the environment, which is determined for one national concentration per compound. Thus, the scattered distribution of concentrations as measured by a monitoring program should be recalculated into a "national concentration", which can be used as input concentration for F_u-assessment. This "recalculation" needs further definition.

Alternatively, field concentrations can be *calculated* by model-simulations of each step in the causality chain:

- production and use of a compound cause emissions (to different environmental compartments);
- an emitted compound will be distributed through and between different compartments leading to compartment-specific concentrations.

Such an approach is now applied for PEC-assessment within the model USES (Uniform System for Evaluation of Substances) (RIVM *et al.*, 1994). The model is able to calculate PEC/PNEC ratios based on input parameters with respect to each step within the causality chain.

USES seems to be a suitable tool for concentration assessment. Main advantage is that concentrations can be calculated for most compounds for which production or emission quantities and some physico-chemical data are available. This implies that I_{tox} can be calculated for many compounds, provided that assessment of the sensitivity distribution functions for these compounds is possible. Main disadvantage of USES-application for concentration assessment is the absence of sufficient input data for many compounds. This implies that many assumptions have to be made, causing uncertainty in the concentration estimates.

3.1.2. Estimation of the sensitivity distribution function

As stated in 1.1.2., I_{tox} is based on the sensitivity distribution of species tested under laboratory conditions. This sensitivity is expressed in terms of NOECs. For each compound, NOECs are collected for as many species as possible, and a logistic distribution function is estimated for the log-values of these data. This method is in line with the present method of MPC-derivation in the Netherlands which is based on an idea by Kooijman (1987), altered by Van Straalen & Denneman (1989), and elaborated by Aldenberg & Slob (1993).

The principle of the MPC-derivation is determination of the 5th percentile of the logistic frequency distribution of the log(NOEC)s, *i.e.* determination of the concentration which exceeds the NOEC of no more than 5% of all species. This is illustrated in Figure 3.1, which shows the actual frequency distribution of (the ^{10}logs of) 19 aquatic NOECs determined under laboratory conditions for γ-hexachlorocyclohexane (Table 4.4). The solid curve from Figure 3.1 shows the calculated logistic probability density function for the log(NOEC)s of Table 4.4. This curve was calculated using the program ETX 1.3A (Aldenberg, 1993). In Figure 3.1, the MPC is indicated as a cut-off value: at the MPC 5% of the species tested in laboratory experiments are exposed to concentrations higher than their NOEC.

ETX estimates the parameters of the logistic and the normal distribution of the log(NOEC)s, *i.e.* it calculates an estimate for the location parameter for the distribution (α and μ, respectively), and an estimate for the scale parameter of the distribution (β and σ, respectively). Characteristics of both distribution functions are given in Appendix *II*.

For many compounds, few NOECs are available as input data for ETX. When ETX is used for criteria setting (*i.e.* MPC-derivation as 5th percentile of the logistic distribution of the log(NOEC)s), an arbitrary minimum number of 4 NOECs is set. With less than 4 NOECs ($n<4$), MPCs are derived by application of assessment factors on toxicity data. In other words, when $n<4$, MPCs are no longer based on the frequency distribution of the log(NOEC)s. It should be realized, however, that up to a minimum of 2 input NOECs, unbiased estimates can be calculated by ETX for both the location and the scale parameters of the log(NOEC) frequency distribution. Therefore, it was decided in the present study that a minimum number of only 2 NOECs is accepted to estimate the logistic frequency distribution by means of ETX[3].

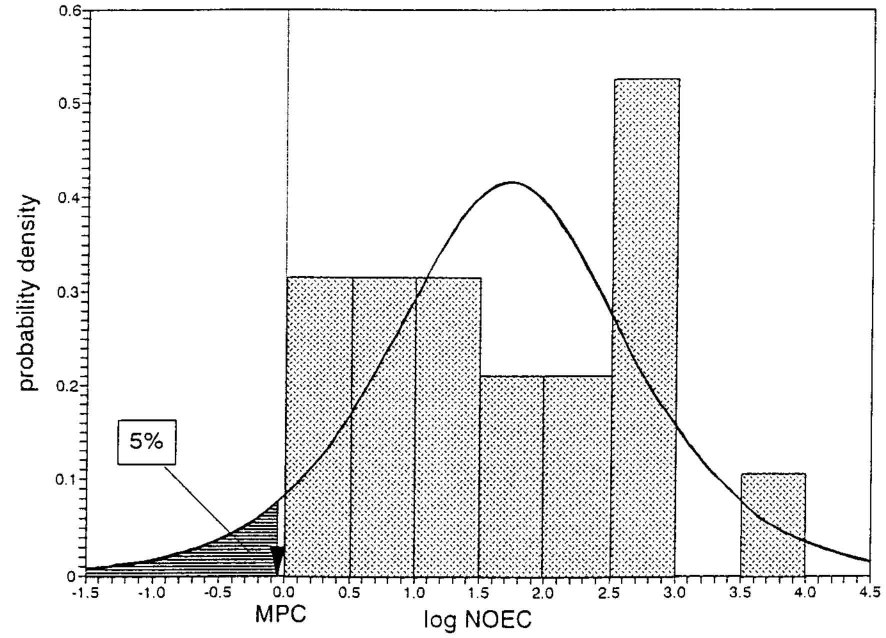

Figure 3.1: Actual (bars) and calculated (solid line) frequency distribution of 19 aquatic log(NOEC)s of γ-hexachlorocyclohexane. The calculated MPC (*i.e.* the 5th percentile of the NOEC distribution function) is indicated as well.

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[3] Two alternative methods have been proposed to estimate a log(NOEC) frequency distribution when less than 4 NOECs are available. In Appendix *III* of this report, a method has been worked out which is based on the same assessment factors as used in MPC derivation in case $n<4$. Luttik and Aldenberg (1995) proposed another method which is based on application of the average σ of related compounds with more than 4 NOECs available. Despite the fact that both alternative methods can cope with compounds for which only one NOEC is available ($n=1$), they are not recommended for the purpose of I_{tox}-calculation due to their disputable theoretical grounds.

3.1.3. Actual F_u-assessment

In the process of deriving environmental quality objectives, the 5th percentile of the log(NOEC) distribution is calculated and regarded as the MPC (Figure 3.1). The new perspective of the present study, is to apply the inverse method: from the NOEC-distribution function the fraction of species is determined for which the (laboratory) NOEC is exceeded at the (estimated) field concentration. This fraction is equal to the surface-area under the curve (as illustrated in Figure 3.1 at MPC), but can also be read directly from the y-axis in a cumulative frequency distribution function, as shown in Figure 3.2. The actual, empirical cumulative frequency of NOECs has also been shown in Figure 3.2. This is done by ranking the 19 NOECs of Table 4.4. Next, a cumulative frequency of 1/19 (=0.053) is attributed to the first (lowest) log(NOEC)=0.029, of 2/19 (=0.105) to the second log(NOEC)=0.342, *etcetera*. Plotting the calculated cumulative distribution function in combination with the cumulative number of actual NOECs demonstrates the accuracy of the fit (Figure 3.2).

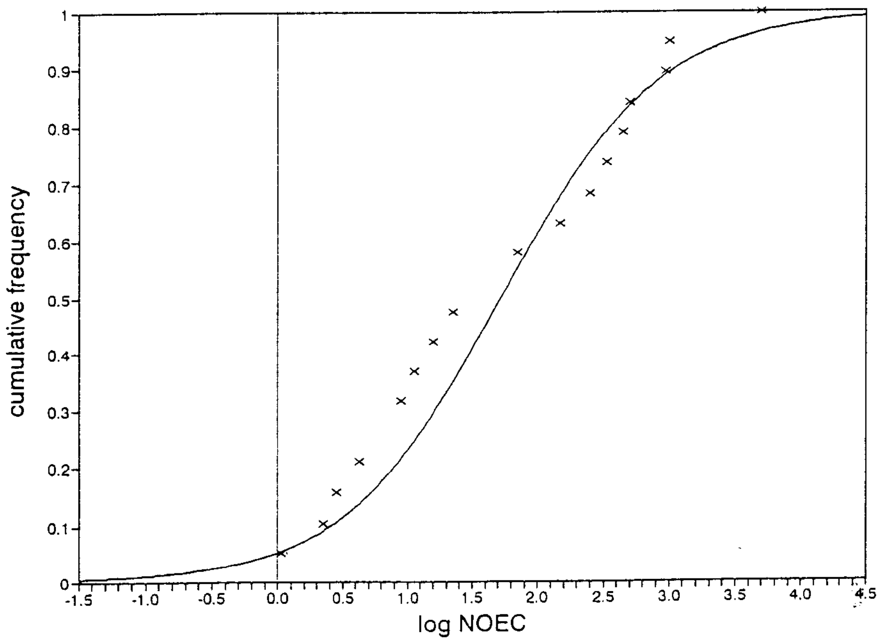

Figure 3.2: Actual (×) and calculated (solid line) cumulative distribution of the 19 aquatic log(NOEC)s shown in Figure 3.1.

F_u is easily estimated by calculating the outcome of the cumulative logistic distribution function (defined by its parameters α and β) at a given field concentration C:

$$F_u(C) = [1 + e^{\frac{-(\ln(C) - \alpha)}{\beta}}]^{-1}.$$

F_u may also be read graphically from the y-axis of the cumulative distribution plot.

3.2. Principles of F_u-aggregation

As defined in 1.1.2., I_{tox} is aggregated out of all compound-specific F_u-values. The indicator should be interpreted as the fraction of the species for which the NOEC is exceeded by at least one of the compounds present within a certain area (F_{uTOTAL}). Within this study, two different principles of F_u-aggregation are worked out. Both principles and a final algorithm to aggregate F_u's are described in separate paragraphs.

3.2.1. Concentration addition

The first method for F_u-aggregation is based on a method commonly used to determine the combination effects in *single-species* toxicity experiments of multiple compounds showing Simple Similar Action (SSA). SSA is characterized by (Placket & Hewlett, 1952):

- same mode of action (primary site of action). Compounds with the same primary site of action show parallel (log)dose-response curves.
- no interaction between the different compounds, *i.e.* the biological action of one compound is not influenced by another compound.

The combined effect of multiple compounds showing SSA is calculated by concentration addition. This method consists of 2 steps:

1 Divide the concentrations of the individual compounds by their compound specific $E(L)C_{50}$, in order to scale the concentrations into Toxic Units (TU), with 1 TU being defined as the concentration causing 50% effect (mortality). By scaling the concentrations into TU, the parallel (log)dose-response curves will merge into one uniform (log)TU-response curve.

2 Calculate the sum of the TU's and read the combination effect from the log(TU)-response curve. Summation of the scaled concentrations is allowed since all compounds affect the same primary site of action and therefore may be regarded as dilutions of each other.

It is proposed to apply concentration addition (developed for single species situations) as a method to aggregate F_u's (based on toxicity data of multiple species). In this context, the cumulative log(NOEC) frequency distribution (*e.g.* Figure 3.2) is regarded as a (log)dose response curve for all species tested under laboratory conditions, comparable to the (log)dose-response curve for a single species. Further application of the concept of concentration addition is also comparable to the single species situation described above, *i.e.*:

1 Divide the NOECs of the separate compounds by their compound specific HC_{50}, in order to scale the concentrations into Hazard Units (HU), with 1 HU being defined as the standardized concentration at which the NOEC is exceeded for 50% of all species tested under laboratory conditions.

2 Calculate the sum of HU and read the aggregated F_u ($F_{u\mathrm{TOTAL}}$) from the frequency distribution function of the log(NOEC')s (NOEC' in terms of HU).

In the case of single species toxicity testing, concentration addition is restricted to compounds which meet the characteristics of SSA. Therefore, concentration addition may not be copied simply to the multiple species situation, but should only be applied when the same requirements are met, *i.e.* same mode of action and no interaction. However, the idea of "same mode of action" or "same primary site of action" is difficult to interpret in the multiple species situation. In contrast with the single species situation, this idea no longer only refers to the mode of action of the different compounds, but also to the response of the different species. In other words, the idea of "same mode of action" should hold not only *between* different log(NOEC) frequency distributions (*i.e.* between different compounds), but also *within* a single log(NOEC) frequency distribution (*i.e.* between different species for the same compound).

Example 1: considering the group of hydrophobic inert compounds, each compound has a similar, non-specific mode of action, called narcosis. The potential of such a compound to induce a narcotic effect depends entirely on its hydrophobicity (Verhaar *et al.*, 1992). Thus, all compounds belonging to this group indeed show the same mode of action. This means that the idea of the "same mode of action" is valid *between* different log(NOEC) frequency distributions. Scaling into HU can be interpreted as a correction for the differences in hydrophobicity, which are responsible for differences in toxicity. Furthermore, since narcosis is a non-specific mode of action, no specific receptors are needed by an organism to respond to a hydrophobic inert compound. This means that all species *within* a single log(NOEC) frequency distribution show the same effect.

Example 2: considering the group of organophosphate (OP) insecticides, each compound has a similar, specific mode of action, called acetylcholinesterase (Ache) inhibition. Thus, all compounds belonging to this group indeed show the same mode of action and may be regarded as dilutions of each other. This means that the idea of "same mode of action" is valid *between* different log(NOEC) frequency distributions. However, since OP insecticides induce such a specific mode of action, a specific receptor (Ache-system) is needed by an organism to respond to an OP insecticide. Species which lack this receptor, are not sensitive to the specific compound and will only be affected at very high concentrations due to the narcotic potency, which is typical for every organic compound (minimum toxicity, Verhaar *et al.*, 1992). This means that the species *within* a single log(NOEC) frequency distribution do not show the same effect. Furthermore, the logistic frequency distribution of the log(NOEC)s of a compound with a specific mode of action is much flatter than for a narcotic compound, due to the big difference in sensitivity for the first compound within a single distribution curve. Although the logistic distribution functions are parallel for compounds which show the same specific mode of action, it may even be argued that the log(NOEC) distribution of a specific compound should be described by a bimodal function (species are sensitive or not) rather than by a logistic function.

Now, consider two compounds which are supposed to have the same mode of action between and within separate log(NOEC) frequency distributions. In theory, these curves are supposed to have parallel courses, but in practice, an "average curve" has often to be calculated. In Figure 3.3, two logistic cumulative frequency distributions of the log(NOEC)s are shown for two compounds that are both considered to belong to the group of hydrophobic, inert organic materials (narcotics). From Figure 3.3, it is obvious that both curves have different slopes, but the hypothesis H_0: $\sigma_A^2=\sigma_B^2$ (log-normal distribution) is not significantly rejected. In Figure 3.4, the dashed lines show the same distributions of Figure 3.3 after standardization of the concentrations into HU. By definition, the curves cross at concentrations of log(NOEC)=0. The "average" NOEC frequency distribution function (solid line) is calculated by running the ETX-program on the combined set of the standardized NOECs (HU) of both compounds. Again, by definition an average α is calculated of $\alpha=0$ and an average β_{AB} is calculated with $\beta_{\min} < \beta_{AB} < \beta_{\max}$.

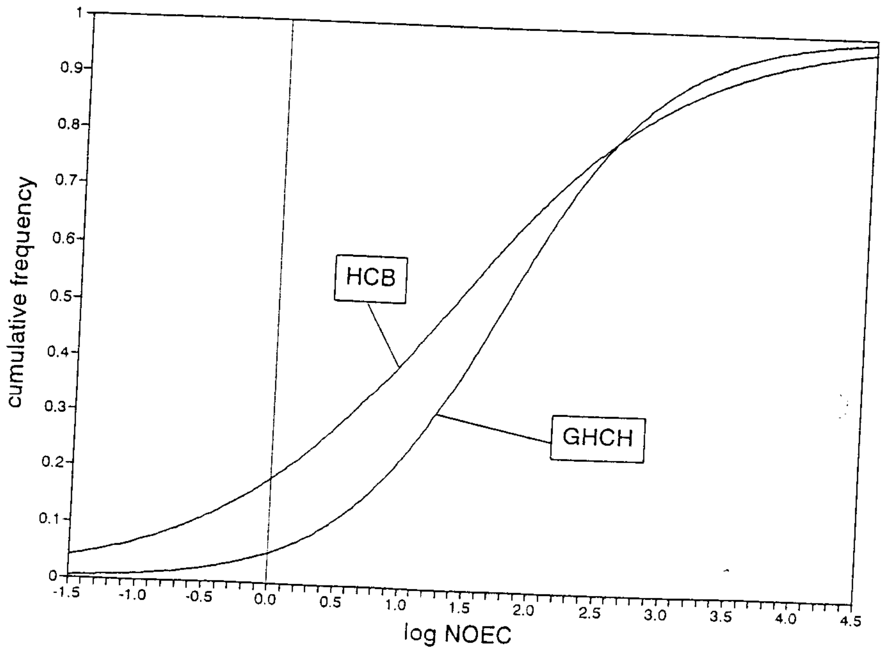

Figure 3.3: Calculated cumulative frequency distributions of aquatic log(NOECs) of γ-hexachlorocyclohexane (GHCH) and hexachlorobenzene (HCB). Both compounds are considered to be narcotics.

The "average" NOEC distribution function is estimated by dividing the NOECs by their compound-specific HC_{50}, which is calculated as $HC_{50}=10^{\hat{\alpha}}$, where $\hat{\alpha}$ is the median of the log(NOEC)s. Thus, the uncertainty of $\hat{\alpha}$ is not taken into account: $\hat{\alpha}$ is used as a constant.

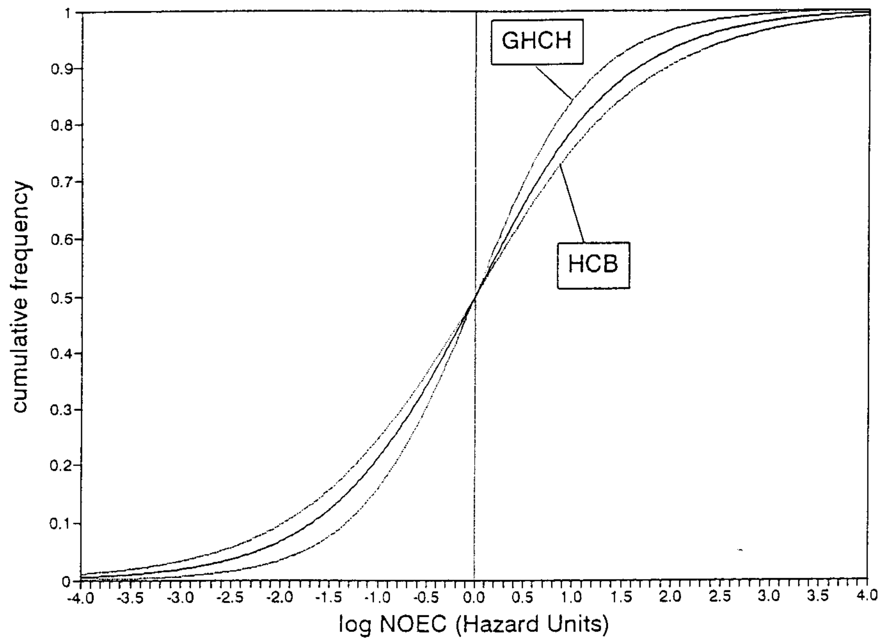

Figure 3.4: Average cumulative frequency distribution for aquatic log(NOEC')s of GHCH and HCB (solid line), calculated after standardization of NOECs into Hazard Units. The dashed lines indicate the original frequency distributions of Figure 3.3 after standardization.

Based on this objection, a second method of concentration addition is now under development, which is based on a multivariate non-linear regression on the NOECs of compounds with the same mode of action. For this purpose, the logistic cumulative frequency distribution of the log(NOEC)s has been transferred into a function expressed in linear (untransformed) concentrations:

$$F_u(C) = \frac{1}{1 + e^{\frac{-(\log(C)-\alpha)}{\beta}}} = \frac{(\frac{C}{a})^b}{1 + (\frac{C}{a})^b}$$

where C=concentration, $a=10\alpha$ and $b=(\beta^{*}\ln 10)^{-1}$ (Appendix *IV*). Next, a multivariate non-linear regression is performed on the NOECs. This multivariate regression is based on the fact that the log(NOEC) frequency distributions of these compounds share the same scale-parameter (β or b) but have their own specific mlocation parameter (HC_{50}, α or a). For example, when concentration addition is applied for two compounds A and B, this leads to the following bivariate non-linear regression:

$$F_{u_{AB}}(C_A,C_B) = \frac{(\frac{C_A}{a_A}+\frac{C_B}{a_B})^b}{1 + (\frac{C_A}{a_A}+\frac{C_B}{a_B})^b}$$

Obviously, this regression function may be extended for >2 compounds into a multivariate non-linear regression. An example of a bivariate case has been worked out in chapter 4.
In summary, the method of concentration addition from *single species* toxicity testing may be modified to determine an aggregated F_u for compounds with the same mode of action. Two modifications are proposed, *i.e.*

1. the aggregated F_u is determined for the sum of the concentrations (in terms of Hazard Units) from an "average" log(NOEC) frequency distribution;
2. the aggregated F_u is determined for each compound-specific concentration from a multi-dimensional surface which is determined by a multivariate non-linear regression.

Both methods are illustrated in chapter 4.

3.2.2. Effect addition

The second method for F_u-aggregation is based on a similar method commonly used to determine the combination effects in *single-species* toxicity experiments of multiple compounds showing Independent Action, which is characterized by (Placket & Hewlett, 1952):

- different modes of action (primary sites of action). Compounds with the same primary site of action do not necessarily show parallel (log)dose-response curves.
- no interaction between the different compounds, *i.e.* the biological action of one compound is not influenced by another compound.

The combination effect of multiple compounds showing Independent Action is calculated by effect addition. For sake of simplicity, this is worked out first for a case with 2 compounds, A and B, with concentrations C_A and C_B. Let us consider that testing of the compounds separately in a single species experiment results in fraction affected organisms of the test population of E_A=0.20 and E_B=0.35 at C_A and C_B, respectively.

Before effect addition in the single species situation may be applied, the "tolerance correlation" (r) of a test population should be known. Tolerance correlation is actually the rank correlation between the sensitivity of the individual organisms for separately applied compounds. For a single species experiment, Könemann (1981) worked out 3 possible values of tolerance correlation:

1. **r=1**: the part of the test population which is most sensitive to compound A is also most sensitive to compound B. In our example, the combination effect may be attributed completely to the compound responsible for the largest effect, *i.e.* the combination effect E_{AB} can be calculated as $E_{AB}=E_B=0.35$. This is explained by the fact that the part of the population which will be affected by compound A already has been affected by compound B, which causes the largest effect. Generally, for r=1 E_{AB} is defined as $\boldsymbol{E_{AB} = E_{max}}$.
2. **r=-1**: the part of the test population which is most sensitive to compound A is least sensitive to compound B. In our example, the combination effect is calculated by addition of both E_A and E_B, *i.e.* $E_{AB} = E_A+E_B = 0.20+0.35 = 0.55$. This is explained by the fact that the part of the population which is affected by compound A is not the part of the population that is affected by compound B. Generally, for r=-1, E_{AB} is defined as $\boldsymbol{E_{AB} = E_A + E_B}$, with a maximum of $E_{AB}=1.0$.
3. **r=0**: sensitivity to compound A is not related to sensitivity to compound B. In this case, the combination effect (E_{AB}) is calculated as $\boldsymbol{E_{AB} = E_A + E_B - (E_A \cdot E_B)}$. This is explained by the fact that the probability for an organism to be affected by compound A and compound B is P(A) and P(B), respectively. Next, calculation of the combination effect, is actually equal to calculation of the combination probability of two non-excluding processes, *i.e.* the probability that an organism is affected by compound A and/or B, which is defined as $\boldsymbol{P(A \cup B) = P(A) + P(B) - P(A \cap B)}$ where $P(A \cap B) = P(A) \cdot P(B)$ (when r=0) and E_A and E_B are the best estimates for P(A) and P(B), respectively. In our example, $E_{AB} = 0.20+0.35-(0.20 \cdot 0.35) = 0.48$.

   The equation $\boldsymbol{E_{AB} = E_A + E_B - (E_A \cdot E_B)}$ can also be deduced in a different way making use of the complement of the effect. Consider the non-affected part of the test population (Q) as the performances after exposure to A, B or a combination of both, *i.e.* $Q_A = 1-E_A$, $Q_B = 1-E_B$ and $Q_{AB} = 1-E_{AB}$. The combination performance is then calculated by multiplication of the separate performances ($Q_{AB} = Q_A \cdot Q_B$). This is explained by the fact that separate exposure to compound A and B results in a performance Q_A and Q_B, respectively. Combined exposure to compound A and B results in a combined performance of a part Q_A of Q_B, *i.e.* $Q_A \cdot Q_B$. In our example, $Q_A = 1.0-0.20 = 0.80$, $Q_B = 1.0-0.35 = 0.65$ and $Q_{AB}=0.52$. $E_{AB} = 1-Q_{AB} = 1-0.52 = 0.48$ which is the same value as found above. Similarity between both approaches can be seen easily when substituting the separate performances as complement of the separate effects:
   $Q_{AB} = 1-E_{AB} = Q_A \cdot Q_B = (1-E_A)(1-E_B) = 1-E_A-E_B+E_A \cdot E_B$. Solving this equation leads to $\boldsymbol{E_{AB} = E_A + E_B - (E_A \cdot E_B)}$.

It should be realized, that the equation $\boldsymbol{P(A \cup B) = P(A) + P(B) - P(A \cap B)}$ is a general rule, which holds not only when r=0, but for all combination probabilities of non-excluding processes. In our example, when r=1, $P(A) = P(A \cap B)$, which leads to $P(A \cup B) = P(B)$. When r=-1, $P(A \cap B)=0$, which leads to $P(A \cup B) = P(A) + P(B)$.

In summary, the combination effect (E_{AB}) has

- a minimum value of ($E_{AB}=E_{max}$) at $r=1$;
- a maximum value of (E_A+E_B) at $r=-1$ (obviously E_{AB} has a maximum value of ($E_{AB}=E_A+E_B=1$));
- an intermediate value of ($E_{AB} = E_A+E_B - E_A \cdot E_B$) at $r=0$. This value is closer to the maximum than to the minimum value.

In other words, pure addition of effects is only performed in case $r=-1$ and ($E_A+E_B \leq 1$). In all other cases, application of the concept of effect addition leads actually to less than addition.

In our example, the combination effect varies in the range 0.35 to 0.55, whereas for $r=0$ a combination effect was calculated of 0.48. These values provide some indication for the combination effect in case r is not equal to -1, 0 and 1. In this case, the exact combination effect may be calculated by a complex equation described by Placket & Hewlett (1948).

Let us now consider the case that $r_{AB}=-1$ and a third compound C is introduced, with $r_{AC}=-1$. In this case, $r_{BC}>0$, since it is impossible that a population shows complete opposite sensitivities to compound A and C and to compound B and C, whereas it shows also complete opposite sensitivity to compound B and C. Therefore, Könemann (1981) concludes that "the concept of negative correlation has no application for mixtures of many chemicals. For these mixtures the correlation coefficient r can vary from 0 to +1."

In case of more than 2 compounds, it is not clear how to calculate the combination effect, since each combination of compounds has its own tolerance correlation coefficient, within the range $0 \leq r \leq 1$. No procedure was found in literature to account for these mutual correlations. For sake of simplicity, the idea is adopted here that all combinations of compounds for which effect addition is applied, have a tolerance correlation of $r=0$. As shown above in the two compound example, the calculated combination effect at $r=0$ is closer to the maximum than to the minimum value, and may thus be considered as a worst case for effect addition of more than two compounds. This is acceptable, considering the finding that in practice the "joint effect" is often underestimated by effect addition. Joint effects often show a tendency to concentration addition, even when the requirements for SSA are not met and the underlying mechanistic processes are not well understood (Könemann (RIVM), personal communication).

It is proposed to apply the method of effect addition (developed for single species situations) to aggregate F_u's (based on toxicity data of multiple species). In this context, the following assumptions have been made:

- the cumulative log(NOEC) frequency distribution may be regarded as a (log)dose-response curve for all species tested;
- F_{uA} is regarded as the effect E_A of compound A on all species;
- F_{pA} is the complement of F_{uA} and is regarded as the performance Q_A of all species, *i.e.* $F_{uA} = 1- F_{pA}$;
- tolerance correlation $r_{AB}=1$ means: species which are most sensitive to compound A are also most sensitive to compound B;
- tolerance correlation $r_{AB}=-1$ means: species which are most sensitive to compound A are least sensitive to compound B;
- tolerance correlation $r_{AB}=0$ means: species sensitivity to compound A is not related to sensitivity to compound B.

As argued above, calculation of F_uTOTAL is worked out for the case that no tolerance correlation is found ($r=0$), *i.e.* no relationship is found between species sensitive to compound A and species sensitive to compound B. F_uTOTAL is calculated as **F_uTOTAL = $F_{uA}+F_{uB}-(F_{uA} \cdot F_{uB})$**.

When a third compound is considered, this formula becomes quite complicated:
$F_u\text{TOTAL} = F_{uA}+F_{uB}+F_{uC} -(F_{uA} \cdot F_{uB}) -(F_{uA} \cdot F_{uC}) -(F_{uB} \cdot F_{uC}) +(F_{uA} \cdot F_{uB} \cdot F_{uC})$.
These complex calculations can be avoided, when F_uTOTAL is calculated by means of multiplication of the complements of the F_u's, comparable to the multiplication of performances in the single species situation. Therefore the "Fraction protected" (F_p) is defined as $F_p = 1-F_u$, whereas F_pTOTAL is calculated as

$$F_p tot = \prod_i F_{p_i}$$

which leads to

$$F_u tot = 1 - F_p tot = 1 - \prod_i (F_{p_i}) = 1 - \prod_i (1 - F_{u_i}).$$

In contrast to the situation of concentration addition, no "average" NOEC-distribution curve has to be determined when effect addition is applied, since F_u is determined for each compound separately from its own specific NOEC frequency distribution before these separate F_u's are aggregated to a F_uTOTAL.

Again, two methods are available to determine the compound-specific NOEC frequency distributions (see Appendices *II* and *IV*). It is decided to apply the same method of curve fitting as used for the narcotics, *i.e.*

1. the compound-specific α_i and β_i are estimated by means of ETX (NOECs are log-transformed) when ETX has also been applied for determination of the "average" NOEC frequency distribution of the narcotic compounds;
2. the compound-specific a_i and b_i are estimated by means of a non-linear regression (NOECs are not transformed) when the compound-specific a_i and the shared $b_{narcotics}$ have also been determined by means of a multivariate non-linear regression on the combined data sets of all narcotics.

E.g., when effect addition is applied for two compounds A and B, F_{uAB} is calculated as $F_{uAB} = 1\text{-}F_{pAB} = 1\text{-}(F_{pA} \cdot F_{pB}) = 1\text{-}\{(1\text{-}F_{uA}) \cdot (1\text{-}F_{uB})\}$.

Ad 1: When ETX is applied, α_i and β_i are determined for each compound individually, before F_{uAB} is calculated:

$$F_{u_{AB}}(C_A,C_B) = 1-[1-(1+e^{\frac{-(\ln(C_A)-\alpha_A)}{\beta_A}})^{-1}]\cdot[1-(1+e^{\frac{-(\ln(C_B)-\alpha_B)}{\beta_B}})^{-1}]$$

where x=log(concentration).

Ad 2: When multivariate non-linear regression is applied, F_p can be calculated as

$$F_{p_i} = 1-F_{u_i} = 1-\frac{(\frac{C_i}{a_i})^{b_i}}{1+(\frac{C_i}{a_i})^{b_i}} = \frac{1}{1+(\frac{C_i}{a_i})^{b_i}}$$

Since no NOECs are available of combinations of the two compounds (*i.e.* $NOEC_{AB}$ is a combination of (C_A,C_B)), the aggregated F_{uAB} is only based on combinations of input concentrations like $NOEC_{AB} = (C_A,0)$ and $NOEC_{AB} = (0,C_B)$. Therefore, it makes no difference whether the combination function for compound A and B is derived by combination of the univariate regression analyses on the NOECs of the individual compounds A and B, or by a multivariate regression analysis on the combined data set of NOECs of compound A and B. Both approaches lead to the following bivariate non-linear regression:

$$F_{u_{AB}}(C_A,C_B) = 1-F_{p_{AB}}(C_A,C_B) = 1-\frac{1}{[1+(\frac{C_A}{a_A})^{b_A}]\cdot[1+(\frac{C_B}{a_B})^{b_B}]}.$$

In summary, the method of effect addition from *single species* toxicity testing may be modified to determine an aggregated F_u for compounds with different modes of action.

Within this method it is important to know the tolerance correlation, which is difficult to determine. Furthermore, it is not quite clear how to aggregate F_u's with a tolerance correlation $0<r<1$. Finally, multivariate non-linear regression may also be used to determine the combination effect by means of effect addition. In this case, it makes no difference whether the univariate distribution curves are fitted separately before aggregating or the multivariate surface area is fitted on a combined set of input NOECs (see chapter 4).

3.2.3. Proposal for F_u-aggregation

The following algorithm is proposed for F_u-aggregation:

1 Select the hydrophobic, inert organic substances out of all compounds present in a certain area;

2 Use concentration addition to aggregate the F_u's of all compounds belonging to this selection. Both methods described in 3.2.1. (*i.e.* concentration addition in terms of HU and by means of multivariate non-linear regression) may be used for this purpose.

3 Use effect addition to aggregate the F_u of the group of hydrophobic inert organic substances with the F_u's of all other compounds, *i.e.*

$$F_{u_{TOTAL}} = 1 - F_{p_{TOTAL}} = 1 - [F_{p_{\Sigma(narcotics)}} \cdot \prod_i F_{p_i}] = 1 - [(1 - F_{u_{\Sigma(narcotics)}}) \cdot \prod_i (1 - F_{u_i})]$$

where i is an individual compound with a specific mode of action, and $F_{p\Sigma\mathrm{narcotics}}$ and $F_{u\Sigma\mathrm{narcotics}}$ are the F_p and F_u, respectively, for all narcotic compounds after concentration addition.

Several possible values for r should be taken into consideration when effect addition is applied.

The algorithm above is based on

- the principles of the *single species* situation, *i.e.* Simple Similar Action is required for concentration addition and Independent Action is required for effect addition;
- the consequences of the examples in 3.2.1, *i.e.* with respect to log(NOEC) frequency distributions (considered as (log)dose-effect curves) the idea of "same mode of action" means not only the same mode of action between different compounds, but also between different species for the same compound.

4. TEST CALCULATION

In this chapter, a test calculation is described in which F_u's are aggregated for 5 compounds. F_u-aggregation is performed separately for three environmental compartments, *i.e.* separate aggregated F_u's are calculated for surface water, soil and sediment. Considering the fact that the aggregated F_u's are only calculated for five compounds, they will be denoted as F_{uTOTAL} rather than I_{tox}, which suggests that toxicity data on many compounds are included.

4.1. Selection of compounds

Selection of the compounds was mainly based on the high availability of data with respect to their emission(s), environmental concentrations and toxicity. If F_u-aggregation turned out to be non-realizable for 5 compounds of which many data are available, the applicability of this method for less known compounds should be reconsidered.

The selected compounds are γ-hexachlorohexane (lindane) (GHCH), pentachlorophenol (PCP), hexachlorobenzene (HCB), cadmium (Cd) and copper (Cu). All five compounds are prioritary substances in Dutch environmental policy (Janus *et al.*, 1994), which are proposed to be reported in the Environmental Outlooks 1997 (Slooff, 1994). Their application, production volume, emissions and environmental concentrations are described in so-called "Integrated Criteria Documents". Furthermore, sufficient toxicity data are available for these compounds to calculate NOEC frequency distribution functions by means of ETX (Van de Plassche, 1994) and finally application of USES is possible for the organic compounds GHCH, HCB and PCP (Gingnagel & Vermeire, 1995).

4.2. Concentration assessment

As described in 3.1.1., input concentrations may have been either calculated or measured. Both options are worked out within this test calculation.

4.2.1. Calculated concentrations

Concentrations were calculated by USES. Most input parameters were obtained from Gingnagel & Vermeire (1995). In contrast with the latter, (regional) environmental concentrations were based on emission data obtained from the "Integrated Criteria Documents", rather than on the tonnage of the compound produced. For all five compounds, the emissions and their corresponding calculated regional environmental concentrations are shown in Table 4.1 and 4.2, respectively. Since no sediment and marine concentrations are calculated by USES, F_u-assessment is impossible for these environmental compartments.

USES has been developed originally for organic compounds. In principle, the program is also applicable for heavy metals when vapor pressures are set to a minimum and partition coefficients are known (EC, 1995). Estimates for the coefficients are given in Appendix *V*.

GHCH: Specific physico-chemical and degradation data of the γ-isomer were used to calculate the field concentration. These data were obtained from Table 1.3 and 3.3, respectively of the Integrated Criteria Document for Hexachlorocyclohexanes (Slooff & Matthijsen, 1990). This in contrast to Gingnagel & Vermeire (1995), who used general input parameters for the whole group of hexachlorocyclohexanes. Furthermore, emissions to air, surface water and agricultural soil for **1984** were obtained from Table 2.7 of Slooff and Matthijsen (1990).

HCB: Emissions to air and surface water for **1987** were obtained from the Integrated Criteria Document (p.15) (Slooff *et al.*, 1991), whereas the USES-estimates of emission to waste water and surface water have been maintained. These estimates were based on a production volume of 430 ton per year (1987).

PCP: Emissions to air, surface water and soil for **1987** were obtained from Table 2.9 of the Integrated Criteria Document (Slooff *et al.*, 1990). Based on the main source of PCP-emission (*i.e.* evaporation from impregnated wood) it was decided that the emission to soil goes to the industrially used soil (*i.e.* 1% of the total system area of The Netherlands) (RIVM *et al.*, 1994).

Cd: Emissions to air, surface water, agricultural soil and industrial soil for **1985** were obtained from Figure 2.4 of the Integrated Criteria Document (Ros & Slooff, 1990). Both national and imported Cd-emissions were included in the concentration calculation. Furthermore, DT_{50}s were set at maximum values (*i.e.* the time needed to degrade 50% of the compound either by biotic or abiotic degradation).

Cu: Emissions to air, surface water and agricultural soil for **1985** were obtained from Table 2.16 of the Integrated Criteria Document (Slooff *et al.*, 1987). Based on the main source of Cu-emission (*i.e.* from animal dung) it was decided that the emission to soil goes to the agricultural soil. Moreover, For this source of emission, the updated value of 1987 was used. Again DT_{50}s were set at maximum values.

4.2.2. Measured concentrations

All measured concentrations were obtained from the Integrated Criteria Documents. As argued in 3.1.1., concentrations have often been measured in more than one spot and/or in more than 1 year. Therefore, the concentrations need to be recalculated into a "national average concentration". When concentrations were measured in more than one spot, the geometric average of the concentrations was calculated. When concentrations were measured in more than one spot in more than one year (*e.g.* in river Rhine, Meuse and Schelde), the geographic, geometric average of the concentrations of the last year was considered to be the "national average concentration". The average concentrations are given in Table 4.3.

4.3. NOEC frequency distributions

All NOECs used to calculate the frequency distributions were obtained from Van de Plassche (1994). Secondary poisoning was incorporated into the frequency distributions, by using not only direct NOECs as input parameters, but also indirect NOECs. This method is described by Van de Plassche (1994). In short, this means that each predator NOEC (in [mg/kg food]) is converted into the corresponding concentration in the environmental compartment by means of the equation

$$NOEC_{water;soil} = \frac{NOEC_{bird;mammal} \cdot ccf_{fish;worm}}{BCF_{fish;worm}}$$

where $ccf_{fish;\,worm}$ is a caloric conversion factor and $BCF_{fish;\,worm}$ is a bioconcentration factor. For the aquatic (water→fish→predator) and the terrestrial food chain (soil→worm→predator), fish and worm are considered to be the most critical route respectively, with $ccf_{fish} = 0.32$ and $ccf_{worm} = 0.23$. BCF-values for the five selected compounds were also obtained from Van de Plassche (1994).

As an example, the calculation of the aquatic NOEC frequency distribution of GHCH is described below. From Van de Plassche (1994), 14 direct and 5 indirect NOECs were obtained (Table 4.4). The indirect NOECs were calculated into water concentrations by means of the equation above, with $ccf_{fish} = 0.32$ and $BCF_{fish}(GHCH) = 480$ l/kg. *E.g.*, the NOEC of 25 mg/kg food for *Mus musculus*, was calculated into a water concentration of

$$NOEC_{water} = \frac{NOEC_{M.musculus} \cdot ccf_{fish}}{BCF_{fish}} = \frac{25 \cdot 0.32}{480} = 0.0167\, mg/l = 16.7\, \mu g/l.$$

Table 4.1: Input emission values per selected compound for the environmental compartments. Calculation of the environmental concentrations by means of USES was based on these emission values.

Release to	dimension	GHCH		HCB		PCP		Cd		Cu	
air	[kg/d]	9.041	ICD[1]	0.205	ICD	137.3	ICD	25.2	ICD	189	ICD
waste water	[kg/d]	0	USES[2]	1.178	USES	0	ICD	0	USES	0	USES
surface water	[kg/d]	0.5479	ICD	0.00822	ICD	10.3	ICD	45.2	ICD	697.8	ICD
agricultural soil	[kg/d]	69.86	ICD	0	USES	0	ICD	21.9	ICD	2591	ICD
industrial soil	[kg/d]	0	USES	0.1178	USES	16.4	ICD	278.1	ICD	0	USES
year		1984		1987		1987		1985		1985[3]	

1 From Integrated Criteria Document
2 Calculated by USES
3 Emission to agricultural soil for 1987

Table 4.2: Calculated concentrations of the selected compounds per environmental compartment.

Compartment	dimension	GHCH	HCB	PCP	Cd	Cu
air	[mg/m^3]	$9.533*10^{-8}$	$1.205*10^{-8}$	$1.655*10^{-6}$	$3.364*10^{-19}$	$2.523*10^{-18}$
surface water	[mg/l]	$2.097*10^{-5}$	$1.660*10^{-8}$	$3.709*10^{-6}$	$3.263*10^{-5}$	$4.420*10^{-4}$
porewater	[mg/l]	$3.342*10^{-4}$	$2.561*10^{-6}$	$6.505*10^{-7}$	$2.857*10^{-4}$	$2.953*10^{-2}$
natural soil	[mg/kg]	$1.727*10^{-4}$	$4.155*10^{-5}$	$5.046*10^{-4}$	$1.057*10^{-1}$	$3.076*10^{-1}$
agricultural soil	[mg/kg]	$2.023*10^{-2}$	$5.477*10^{-3}$	$3.496*10^{-4}$	$1.441*10^{-1}$	5.091
industrial soil	[mg/kg]	$1.727*10^{-4}$	$3.244*10^{-2}$	2.826	$1.168*10^{+2}$	$3.076*10^{-1}$

Table 4.3: Measured concentrations of the selected compounds per environmental compartment.

Compartment	dimension	GHCH			HCB			PCP		
		conc.	source in reference	year	conc.	source in reference	year	conc.	source in reference	year
air	[mg/m^3]	$3.3*10^{-7}$	Table 4.12	1985	$4*10^{-6}$	Table 4.8	1982-1983	$7*10^{-7}$	Table 4.9	1988
surface water	[mg/l]	$4*10^{-5}$	Figure 4.1	1985	$1*10^{-3}$	Table 4.4	1988	$3.4*10^{-5}$	Table 4.5	1988
sediment	[mg/kg]	$1.314*10^{-1}$	Table 4.7	1985-1986	$<3*10^{-2}$	page 52	1988	$8.4*10^{-3}$	Table 4.7	1979-1980
natural soil	[mg/kg]	$1.127*10^{-3}$	Table 4.3	1984	$5.6*10^{-4}$	page 47	1984	$4.9*10^{-4}$	Table 4.2	1984
reference		Slooff & Matthijsen, 1990			Slooff *et al.*, 1991			Slooff *et al.*, 1990		

Compartment	dimension	Cd			Cu		
		conc.	source in reference	year	conc.	source in reference	year
air	[mg/m^3]	$<2*10^{-9}$	Figure 3.6	1982-1983	$1.9*10^{-5}$	Table 4.7	1982-1983
surface water	[mg/l]	$3.2*10^{-4}$	page 35	1982	$5.5*10^{-3}$	Table 4.6	1987
sediment	[mg/kg]	<4.4	Table 4.5	1985-1986	$9.3*10^{+1}$	Table 4.5	1985
natural soil	[mg/kg]	$4.3*10^{-1}$	Table 4.1	1983	6.8	Table 4.1	1986
reference		Ros & Slooff, 1990			Slooff *et al.*, 1987		

The logistic frequency distribution function of all log(NOEC)s from Table 4.4 was estimated by means of the ETX program. For the aquatic GHCH, parameter estimates were $\hat{\alpha} = 1.7230$ and $\beta = 0.6002$. The frequency distribution of the actual log(NOEC)s of Table 4.4 is shown above in Figure 3.1 (bars) together with the estimated probability distribution which is defined by $\hat{\alpha}$ and β, *i.e.*

$$f(C) = \frac{e^{\frac{-(\log(C)-1.7230)}{0.6002}}}{0.6002\cdot[1+e^{\frac{-(\log(C)-1.7230)}{0.6002}}]^2}.$$

The cumulative frequency distribution of the actual log(NOEC)s of Table 4.4 is shown in Figure 3.2 (×) together with the estimated cumulative frequency distribution, which is also defined by $\hat{\alpha}$ and β, *i.e.*

$$F(C) = [1+e^{\frac{-(\log(C)-1.7230)}{0.6002}}]^{-1}$$

Table 4.5 shows the parameter estimates of both the aquatic and terrestrial logistic frequency distributions of log(NOEC)s of the 5 selected compounds together with their 5th percentile, the calculated field concentration (Predicted Environmental Concentration, PEC), the measured field concentration, and the corresponding F_u-values per compound.

Only for the compartments surface water and soil, sufficient toxicity data were available to determine NOEC frequency distributions. For sediments, the F_u-value may be derived from the water F_u-value, assuming equilibrium partitioning, *i.e.*

$$C_{sediment} = 10^{-3}\cdot K_p\cdot c_{water}$$

(with $C_{sediment}$ in mg/kg, C_{water} in μg/l and K_p in l/kg) and assuming equal sensitivity of sediment inhabiting species and aquatic species. Thus, all aquatic NOECs are divided by their K_p-value whereafter a sediment NOEC frequency distribution function is estimated. In practice, this comes down to

$$\alpha_{sediment} = \alpha_{water} - 3 + \log(K_p)$$

and

$$\beta_{sediment} = \beta_{water}.$$

The outcome of this exercise is shown in Table 4.6. Sediment F_u's were only estimated for measured field concentrations, since USES does not calculate sediment concentrations.

Application of the equilibrium partitioning model for the sediment-water system is based on the porewater hypothesis, *i.e.* the magnitude of the effect can be described by the concentration in the pore water of the sediment. This model is not simply transferable to the air-water system. Since no ecotoxicity data are available for the air compartment and risk analysis of air concentrations depends mainly on human toxicity data, this compartment is not taken into consideration (see 5.1.3.).

Table 4.4: Aquatic NOECs for GHCH (Van de Plassche, 1994) and their corresponding input-values for the ETX-estimation of the NOEC frequency distribution function, in terms of log(NOEC) and log(HU); $\log(HU)=\log(NOEC/HC_{50})$ where $HC_{50}=52.85$ μg/l. Indirect NOECs [mg/kg food] were calculated into water concentrations [μg/l water].

NOEC	organism	input NOEC [μg/l]	log input NOEC log [μg/l]	log input NOEC log [HU]
DIRECT [μg/l]				
150	blue algae (fresh water)	150	2.18	0.453
250	green algae (fresh water)	250	2.40	0.675
950	green algae (fresh water)	950	2.98	1.25
500	green algae (fresh water)	500	2.70	0.976
440	protozoa (fresh water)	440	2.64	0.920
330	molluscs (fresh water)	330	2.52	0.796
11	crustaceans (fresh water)	11	1.04	-0.682
4.3	crustaceans (fresh water)	4.3	0.634	-1.09
2.2	insects (fresh water)	2.2	0.342	-1.38
9.1	fish (fresh water)	9.1	0.959	-0.764
2.9	fish (fresh water)	2.9	0.462	-1.26
8.8	fish (fresh water)	8.8	0.945	-0.779
5000	? (salt water)	5000	3.70	1.98
1000	? (salt water)	1000	3.00	1.28
INDIRECT [mg/kg food]				
25	*Mus musculus* (mammal)	16.7	1.22	-0.500
33	*Oryctolagus cuniculus* (mammal)	22	1.34	-0.381
100	*Rattus norvegicus* (mammal)	66.7	1.82	0.101
1.6	*Gallus domesticus* (bird)	1.07	0.029	-1.69
100	*Anas plathyrhynchos* (bird)	66.7	1.82	0.101

Table 4.5: Characteristics of the ETX-estimated logistic frequency distribution functions of the log(NOEC)s of the five selected compounds, in surface water and soil, together with their individual F_u-values at calculated and measured concentrations. Individual F_u-values exceeding 5% are printed bold.

	GHCH	HCB	PCP	Cd	Cu
SURFACE WATER					
α	1.7230	1.3216	2.0506	1.5875	1.3876
β	0.6002	0.8911	0.4726	0.6217	0.2934
n	19	25	26	55	42
HC_5[1] [μg/l]	0.9032	0.0499	4.5617	0.5713	3.3389
PEC [μg/l]	0.0210	0.00002	0.0037	0.0326	0.4420
PEC/HC_5	0.0232	0.0003	0.0008	0.0571	0.1324
Field concentration [μg/l]	0.04	0.001	0.034	0.3162	5.475
F_u(PEC)	0.0034	0.0022	0.00008	0.0071	0.0026
F_u(Field concentration)	0.0055	0.0115	0.0006	**0.0722**	**0.0986**
NATURAL SOIL					
α	0.5844	0.5547	1.2251	0.2888	1.7268
β	0.6383	0.7862	0.3567	0.5098	0.4910
n	7	7	9	17	14
HC_5[1] [mg/kg]	0.0507	0.0174	1.4955	0.0613	1.91
PEC [mg/kg]	0.0002	0.00004	0.0005	0.1057	0.3076
PEC/HC_5	0.0034	0.0024	0.0003	1.723	0.161
Field concentration [mg/kg]	0.0011	0.562	0.486	0.427	6.785
F_u(PEC)	0.0011	0.0019	0.000003	**0.0772**	0.0104
F_u(Field concentration)	0.0039	**0.2643**	0.0132	**0.215**	**0.139**

---

[1] The calculated 5th percentile of the NOEC frequency distribution (HC_5) is equal to the MPC derived by Van de Plassche (1994) for the same NOECs. This is due to the fact that Van de Plassche preferred to derive separate MPCs from direct and indirect NOECs and to define the lowest HC_5 as the MPC (Method I). In the present case, data sets of direct and indirect NOECs were combined and an aggregated HC_5 was estimated (method II). Both methods are described further by Van de Plassche (1994).

Table 4.6: Characteristics of the logistic frequency distribution functions of the log(NOECs) of the five selected compounds in the sediment compartment, together with their individual F_u-values at measured concentrations only. The parameter estimates were derived from the frequency distribution of log(NOEC)s for fresh water organisms. Individual F_u-values exceeding 5% are printed bold.

	GHCH	HCB	PCP	Cd	Cu
SEDIMENT					
α	0.6492	1.7979	1.9268	3.3314	2.7516
β	0.6002	0.8911	0.4726	0.6217	0.2934
HC_5 [mg/kg]	0.0762	0.1493	3.4304	31.6769	77.1954
Field concentration [mg/kg]	0.1314	0.03[1]	0.0084	4.4[1]	93
F_u(Field concentration)	**0.0724**	0.0235	0.0002	0.0131	**0.0648**

[1] worst case concentration

4.4. F_u-aggregation

Based on the classification of Verhaar (1992), GHCH and HCB are both considered to belong to the group of inert hydrophobic compounds. As argued in 3.2.1., both compounds are supposed to have the same, non-specific mode of action, called narcosis. For PCP, Cd and Cu, differences are expected between and within the separate cumulative NOEC frequency distributions with regards to mode of action and receptors. Based on the algorithm proposed in 3.2.3., the F_uTOTAL of the five compounds is calculated by concentration-addition of the narcotic compounds GHCH and HCB and further F_u-aggregation by effect addition, *i.e.*

$$F_{p_{TOTAL}} = F_p(GHCH,HCB) \cdot F_p(PCP) \cdot F_p(Cd) \cdot F_p(Cu)$$

$$= [1 - F_u(GHCH,HCB)] \cdot [1 - F_u(PCP)] \cdot [1 - F_u(Cd)] \cdot [1 - F_u(Cu)]$$

where $F_{u}TOTAL = 1 - F_{p}TOTAL$. The principle of F_u-aggregation has been worked out extensively for the surface water compartment in 4.4.1. and 4.4.2. For the other compartments, only final results will be shown.

4.4.1. Concentration addition

Figure 4.1 shows the frequency distributions of aquatic NOECs for GHCH and HCB. Figure 4.1 confirms the finding $HC_{50}(GHCH) > HC_{50}(HCB)$ (Table 4.5: $\alpha_{GHCH} > \alpha_{HCB}$) and the finding that HCB-NOECs are found in a wider range than GHCH-NOECs (Table 4.5: $\beta_{GHCH} > \beta_{HCB}$). Two different methods are proposed to test whether both curves differ significantly:

1 a test on the significance of differences between the two variances (H_0: $\sigma^2_{GHCH} = \sigma^2_{HCB}$ (Sokal & Rohlf, 1981);

2 a generalized likelihood ratio-test (Crommentuijn, personal communication).

Execution of the first test showed that the shape of both bell-shaped curves did not differ significantly. This is in line with our expectation that compounds with a narcotic mode of action have parallel cumulative frequency distributions. The cumulative S-curves have already been shown in Figure 3.3.

Since the slopes of the separate curves did not differ significantly, the concept of concentration addition was allowed for GHCH and HCB. This implies that

1 the NOECs are recalculated into Hazard Units;

2 an average cumulative distribution function is calculated for the standardized NOECs;

3 the (calculated) field concentrations of both GHCH and HCB are recalculated into Hazard Units whereafter the sum of HU is determined;

4 F_u(GHCH,HCB) is calculated by means of the average cumulative frequency distribution and the (calculated) field concentration.

Ad 1: As defined in 3.2.1., standardizing the NOECs into Hazard Units means dividing the NOECs by the HC_{50}. Despite the dimension of the NOECs (*i.e.* μg/l, mg/kg or HU), the logistic distribution function requires log(NOEC) data as input data. Therefore, the logarithm of the standardized NOEC (log(NOEC')) was calculated by

$$\log(NOEC') = \log(NOEC) - \log(HC_{50}) = \log(NOEC) - \alpha .$$

For GHCH, the log(NOEC')-values have been shown in the last column of Table 4.4. For HCB, exactly the same procedure was followed (data not shown).

Ad 2: By means of ETX, the average logistic frequency distribution was determined for the combined set of log(NOEC')-values for GHCH and HCB (n=44):

$$F_u(GHCH,HCB) = [1 + e^{\frac{-\log(GHCH' + HCB') + 0}{0.7612}}]^{-1}$$

where GHCH' and HCB' are the standardized concentrations of GHCH and HCB, respectively. This cumulative distribution function is shown as the solid line of Figure 3.4. Obviously, the parameter estimates of this "average" frequency distribution have the expected values, *i.e.*

- $\alpha=0$ implies that $HC_{50}' = 10^{\alpha} = 1$. This means that $F_u = 50\% = 0.5$ at a concentration of 1 Hazard Unit, which is the original definition of 1 HU.
- $\beta=0.7612$ which is a value in between the extremes of $\beta(GHCH)=0.6002$ and $\beta(HCB)=0.8911$. The "average" slope can also be seen from Figure 3.4.

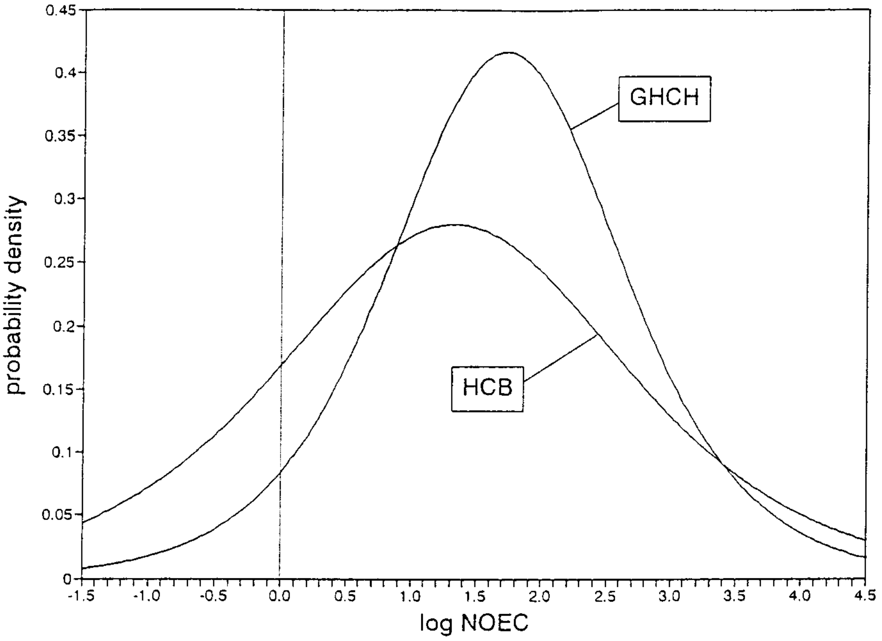

Figure 4.1: Calculated frequency distributions of aquatic log(NOEC)s of GHCH and HCB. Both compounds are considered to be narcotics.

Ad 3: For GHCH, a surface water concentration of 0.02097 μg/l was calculated (Table 4.2). This concentration was standardized as log(GHCH') = log(0.02097) - 1.7230 = -3.4014. For HCB, a surface water concentration of 0.0000166 μg/l was calculated (Table 4.2). This concentration was standardized as log(HCB') = log(0.0000166) - 1.3216 = -6.1015. The sum of the concentrations in terms of HU was determined as
$(GHCH'+HCB') = 10^{-3.4014} + 10^{-6.1015} = 0.00039762$ and $\log(GHCH'+HCB') = -3.4005$.

Ad 4: The standardized sum of calculated concentrations is filled out in the equation above:

$$F_u(GHCH,HCB) = [1 + e^{\frac{3.4005}{0.7612}}]^{-1} = 0.0113$$

The characteristics of the combined NOEC frequency distribution functions of GHCH and HCB for the compartments surface water, natural soil and sediment are shown in Table 4.7.

In 3.2.1., objection was made to the method of estimation of the "average" distribution function applied above, because the uncertainty of $\hat{\alpha}$ is not taken into account when standardizing concentrations into HU's. An alternative method was proposed to apply the principle of concentration addition, *i.e.* the multivariate regression analysis. In this two compound example, a 3D surface area is fitted for the cumulative frequency of combinations of the actual NOECs. Since no data were available on NOEC-combinations, this surface area is only based on input data as ($NOEC_{GHCH}$, 0) and (0, $NOEC_{HCB}$), *i.e.* combinations of NOECs which are situated in the lateral sides of Figure 4.2.

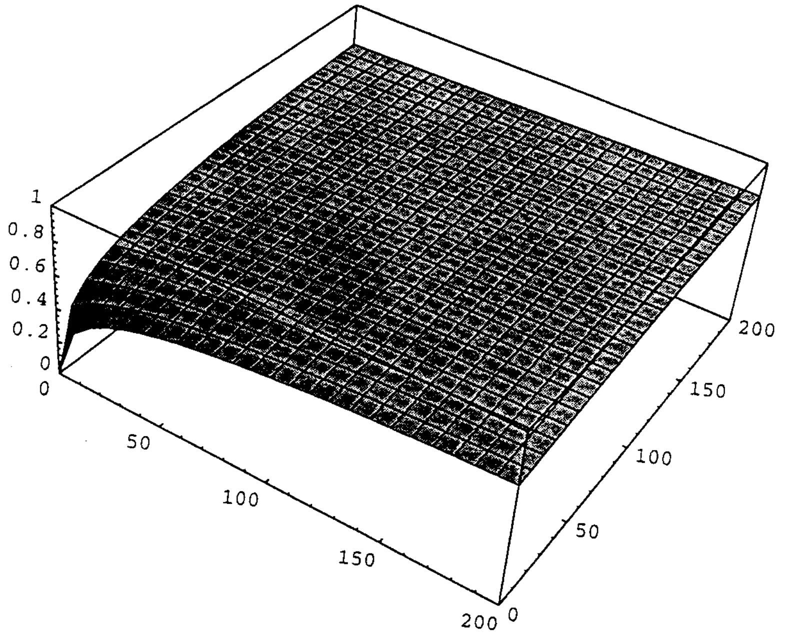

Figure 4.2: Three-dimensional presentation of the surface area which was determined by means of a bivariate non-linear regression on the empirical cumulative frequency distributions of aquatic NOECs of GHCH and HCB, which can be seen in the lateral sides of the 3-D box.

F_u-aggregation by this method is only performed for the surface water compartment. The outcome of this fit on 44 NOECs is

$$F_{u_{GHCH,HCB}} = \frac{\left(\frac{C_{GHCH}}{40.35}+\frac{C_{HCB}}{25.04}\right)^{0.5526}}{1+\left(\frac{C_{GHCH}}{40.35}+\frac{C_{HCB}}{25.04}\right)^{0.5526}}$$

with $R^2=0.975$. By means of this equation, F_u(GHCH,HCB) was calculated for the surface water compartment, *i.e.* F_u(GHCH,HCB) = 0.0151 and F_u(GHCH,HCB) = 0.0219 for the calculated and measured concentrations, respectively (Table 4.5).

Table 4.7: Characteristics of the combined logistic log(NOEC') [HU] frequency distributions of GHCH and HCB for the compartments surface water (see text), natural soil and sediment, together with their F_u-values at calculated and measured concentrations. For all three compartments, HC_{50} is defined as 1 HU.

	GHCH,HCB
SURFACE WATER	
β	0.7612
n	44
HC'_5 [1] [HU]	0.00574
PEC' [HU]	0.000398
PEC'/HC'_5	0.0693
Field concentration' [HU]	0.000805
F_u(PEC')	0.0113
F_u(Field concentration')	0.0169
NATURAL SOIL	
β	0.6570
n	14
HC'_5 [1] [HU]	0.01163
PEC' [HU]	0.0000566
PEC'/HC'_5	0.00486
Field concentration' [HU]	0.1570
F_u(PEC')	0.00155
F_u(Field concentration')	0.227
SEDIMENT	
β	0.7612
n	44
HC'_5 [1] [HU]	0.00574
Field concentration' [HU]	0.0299
F_u(Field concentration')	0.119

[1] See footnote of Table 4.5.

4.4.2. Effect addition

By now, the F_u's for all four mode of actions which were distinguished (*i.e.* F_u(GHCH,HCB), F_u(PCP), F_u(Cd) and F_u(Cu)) are computed by means of the logistic frequency distributions which were determined by means of ETX. As proposed by the algorithm of 3.2.3., these F_u's are further aggregated by effect addition.

This is illustrated in the scheme below:

SURFACE WATER calculated concentrations

F_u(GHCH,HCB) = 0.0113	(Table 4.7)	⇒	F_p(GHCH,HCB) = 0.989
F_u(PCP) = 0.0000762	(Table 4.5)	⇒	F_p(PCP) = 1.00
F_u(Cd) = 0.00707	(Table 4.5)	⇒	F_p(Cd) = 0.993
F_u(Cu) = 0.00263	(Table 4.5)	⇒	F_p(Cu) = 0.997
			⇓
F_uTOTAL = 0.0210 = 2.10%		⇐	F_pTOTAL = 0.979

SURFACE WATER measured concentrations

F_u(GHCH,HCB) = 0.0169	(Table 4.7)	⇒	F_p(GHCH,HCB) = 0.983
F_u(PCP) = 0.000583	(Table 4.5)	⇒	F_p(PCP) = 0.999
F_u(Cd) = 0.0153	(Table 4.5)	⇒	F_p(Cd) = 0.928
F_u(Cu) = 0.0986	(Table 4.5)	⇒	F_p(Cu) = 0.901
			⇓
F_uTOTAL = 0.144 = 14.4%		⇐	F_pTOTAL = 0.856

NATURAL SOIL calculated concentrations

F_u(GHCH,HCB) = 0.00155	(Table 4.7)	⇒	F_p(GHCH,HCB) = 0.998
F_u(PCP) = 0.00000312	(Table 4.5)	⇒	F_p(PCP) = 1.00
F_u(Cd) = 0.0772	(Table 4.5)	⇒	F_p(Cd) = 0.923
F_u(Cu) = 0.0104	(Table 4.5)	⇒	F_p(Cu) = 0.990
			⇓
F_uTOTAL = 0.0882 = 8.82%		⇐	F_pTOTAL = 0.912

NATURAL SOIL measured concentrations

F_u(GHCH,HCB) = 0.227	(Table 4.7)	⇒	F_p(GHCH,HCB) = 0.773
F_u(PCP) = 0.0132	(Table 4.5)	⇒	F_p(PCP) = 0.987
F_u(Cd) = 0.215	(Table 4.5)	⇒	F_p(Cd) = 0.785
F_u(Cu) = 0.139	(Table 4.5)	⇒	F_p(Cu) = 0.861
			⇓
F_uTOTAL = 0.485 = 48.5%		⇐	F_pTOTAL = 0.515

SEDIMENT measured concentrations

F_u(GHCH,HCB) = 0.119	(Table 4.7)	⇒	F_p(GHCH,HCB) = 0.881
F_u(PCP) = 0.000210	(Table 4.6)	⇒	F_p(PCP) = 1.00
F_u(Cd) = 0.0131	(Table 4.6)	⇒	F_p(Cd) = 0.987
F_u(Cu) = 0.0648	(Table 4.6)	⇒	F_p(Cu) = 0.935
			⇓
F_uTOTAL = 0.187 = 18.7%		⇐	F_pTOTAL = 0.813

In the scheme above, for each mode of action the F_p is calculated (from the F_u), whereafter the F_pTOTAL is calculated as the product of the separate F_p's ($r=0$; see 3.2.2.). Finally, F_uTOTAL is calculated as the complement of F_pTOTAL.

As explained in 3.2.2., regression analysis may also be applied to aggregate the F_u's by effect addition. Due to the fact that no NOEC-combinations of different compounds are available, equal parameter estimates will be found at univariate and multivariate regression. In this example, this means that separate fits may be performed for the 4 separate modes of action, or one single fit may be performed on all NOECs together. In other words, for i is (GHCH,HCB), (PCP), (Cd) and (Cu), regression curves are determined either separately:

$$F_{u_i} = \frac{\left(\frac{C_i}{a_i}\right)^{b_i}}{1 + \left(\frac{C_i}{a_i}\right)^{b_i}}$$

or together (see 3.2.2.):

$$F_{u_{TOTAL}} = 1 - \frac{1}{\prod_i \left[1 + \left(\frac{C_i}{a_i}\right)^{b_i}\right]}$$

where

$$\left(\frac{C_{(GHCH+HCB)}}{a_{(GHCH+HCB)}}\right)^{b_{(GHCH+HCB)}} = \left(\frac{C_{(GHCH)}}{a_{(GHCH)}} + \frac{C_{(HCB)}}{a_{(HCB)}}\right)^{b_{(GHCH+HCB)}}.$$

The parameter estimates for a_i and b_i the four modes of action in the surface water compartment are given in Table 4.8.

Table 4.8: Parameter estimates for the multivariate non-linear regression on the cumulative frequency of surface water NOECs.

Mode of action	a	b
(GHCH,HCB)	GHCH 40.35 HCB 25.04	0.5526
PCP	90.44	0.8102
Cd	37.01	0.5659
Cu	21.55	1.463

Finally F_uTOTAL was calculated for the surface water compartment, by means of the equation above and the parameter estimates from Table 4.8, *i.e.* **F_uTOTAL = 0.0367** and **F_uTOTAL = 0.194** for the calculated and measured concentrations, respectively (Table 4.5).

Despite the fact that the non-linear regression equation (Appendix *IV*) was derived from the logistic frequency distribution, the parameters a and b can not be calculated directly from the original α and β, *i.e.* $a=10^{\alpha}$ and $b=1/(\beta*\ln 10)$. This is due to the fact that

- α and β are estimated by ETX which computes compound specific NOEC frequency distributions (*i.e.* α_{A}, β_{A}, α_{B}, β_{B}, *etcetera*). Standardization in HU recalculates each specific α of the narcotic compounds back to zero ($\alpha'=0$), whereas the ß-values are not altered. For the narcotic compounds, an "average" frequency distribution with an average slope ($\beta_{narcotics}$) is determined to enable concentration addition.
- a and b are estimated by a non-linear regression on the empirical cumulative distribution. For narcotics, a surface area is fitted through all the specific frequency distributions, with a compound specific location (*i.e.* a_{A}, a_{B}, *etcetera*), but a similar slope (only one b-value is estimated).

Moreover, the methods of parameter determination differ mathematically.

In summary, separate F_{u}TOTAL-values of the five selected compounds were calculated:

- for different environmental compartments;
- based on calculated and measured concentrations;
- by different methods, *i.e.*
  1. by means of Hazard Units and determination of an "average" frequency distribution;
  2. by non-linear regression on the combined data sets.

The outcome of these various procedures are summarized in Table 4.9, together with the sum of PEC/HC_5-ratios.

Table 4.9: F_{u}TOTAL-values computed at different methods and PEC/HC_5-ratios for the separate compartments, based on calculated and measured field concentrations

Compartment	F_{u}TOTAL (Hazard Units)		F_{u}TOTAL (non-linear regression)[2]		$\Sigma(PEC/HC_5)$[1]
	calculated conc.s	measured conc.s	calculated conc.s	measured conc.s	calculated conc.s
Surface water	0.0210	0.144	0.0367	0.194	0.260
Natural soil	0.0882	0.485			1.889
Sediment[3]		0.187			

1 For (GHCH,HCB), the PEC/HC_5-value of Table 4.7 was used.

2 The non-linear regression method was only applied to the surface water compartment.

3 For the sediment compartment, no concentrations were calculated by USES.

The results from Table 4.9 will be discussed in chapter 5.

5. DISCUSSION

The methods of F_u-calculation and F_u-aggregation as proposed in chapter 3 and worked out in chapter 4 imply some fundamental choices. These choices are defined in 1.2. and are explicitly discussed in the first part of this chapter. The second part focusses on the potency of F_u-assessment and F_u-aggregation as a basis for an environmental quality indicator. The third part of this chapter discusses the outcome of the test calculation.

5.1. Fundamental choices

5.1.1. Chronic *vs* acute toxicity data

Should frequency distributions be based on chronic or acute toxicity data?

In practice, acute environmental effects of toxic compounds occur only in cases of calamity. The environmental policy with respect to the theme of dispersion focusses on compounds which are present continuously at background or increased concentrations. Therefore, MPC-determination is based on frequency distributions of chronic laboratory NOECs (Van de Plassche, 1994), and it is obvious that F_u-assessment is based on exactly the same data. In the case that less than 2 NOECs are available, L(E)C_{50}s can be converted into NOECs by means of the assessment factors which are commonly used for MPC-derivation from acute toxicity data in case less than 4 NOECs are available (Appendix *III*).

5.1.2. Compartments

Should the separate $F_{u\text{TOTAL}}$-values per compartment be aggregated further into a $F_{u\text{TOTAL}}$-value for the total environment?

The maximum level of F_u-aggregation which has been described so far is a $F_{u\text{TOTAL}}$ per environmental compartment, *i.e.* separate $F_{u\text{TOTAL}}$-values are proposed for surface water, soil, sediments, air, (marine environment). A possible approach to generate a $F_{u\text{TOTAL}}$-value for the total environment is to aggregate the separate $F_{u\text{TOTAL}}$-values per compartment into a single overall value. *E.g.*, further aggregation may consist of averaging the $F_{u\text{TOTAL}}$'s per compartment, possibly after application of a weighing factor which is determined by the number of species per compartment or any other factor which indicates the relative importance of the compartments.

The only goal of further aggregation would be to offer the users of I_{tox} a single value which indicates the quality of the total environment with respect to toxic compounds. However, the outcome of such a procedure is hard to interpret in terms of species for which the NOEC determined under laboratory conditions is exceeded under field conditions. Further aggregation leads to a loss of valid information and expressiveness of the indicator. Four or five F_uTOTAL-values for the separate environmental compartments offer an amount of sufficient, compressed information which can easily be overseen and interpreted. There is no need of further aggregation into a F_uTOTAL-value for the total environment.

Finally, the indicator should enable evaluation of the environmental policy. For the compartments soil and water, the environmental policy is mainly based on ecotoxicological risk assessment. On the contrary, risk assessment for the air compartment is exclusively based on human toxicity data. The principle that human toxicity data and ecotoxicity data should not be pooled to indicate the environmental quality (see 5.1.3.), gives rise to another reason not to aggregate the F_uTOTAL-values per compartment into a F_uTOTAL for the total environment.

5.1.3. Ecotoxicity *vs* human toxicity

Should I_{tox} also indicate the environmental quality with respect to human health?

Human risk assessment (RA) is performed at the organization level of the human species, *i.e.* the lethal risk is estimated for each human being individually. This risk should not exceed a defined risk limit. Ecotoxicological RA is performed at the organization level of community structure, *i.e.* the risk of NOEC-exceedance is estimated for all species together. This risk should not be exceeded for more than 5% of all species. Furthermore, no carcinogenic or mutagenic effects are taken into account in ecotoxicological RA, whereas these effects are extremely important within human RA. Based on these different principles in RA procedures, it is proposed that I_{tox} should only give an indication of the environmental quality with respect to the ecosystem and not to human health.

5.1.4. Regional *vs* local

Should I_{tox} be calculated on a continental, a regional or a local scale?

I_{tox} was originally meant to be applied as a national policy performance indicator for the effect of toxic compounds on the environmental quality. This implies that a compartment-specific regional F_uTOTAL-value is calculated for The Netherlands, which is aggregated from F_u-values, which are based on average "national" concentrations.

The I_{tox}-concept is also applicable within the framework of the Global Environmental Outlooks. Based on the same principles for I_{tox}-calculation on the regional (national) scale, a compartment-specific European I_{tox}-value can be calculated, which is based on average European concentrations.

A national or continental concentration per compound can either be calculated (*e.g.* by means of USES) or be determined from measured concentrations. Both calculated and measured concentrations are attended with uncertainty. Uncertainty in calculated concentrations is introduced by the fact that calculations are often based on general assumptions and a minimum set of input data. Uncertainty in measured concentrations is introduced by the fact that valid information will be lost when the spatially differentiated concentrations obtained from monitoring programs are finally aggregated (averaged) into one singular national concentration.

In principle, it should be possible to aggregate the potential risk into I_{tox} of every toxic compound which is emitted to the environment. Based on a minimum set of input data, field concentrations can be calculated for almost every compound. Measured concentrations are only available for compounds which are recognized for their possible risk to man or the environment, *e.g.* the "attention substances". Therefore, it is proposed that I_{tox} should be based on calculated concentrations of as many compounds as possible.

For the sake of clarity, the word I_{tox} is reserved only for the aggregated F_uTOTAL of as many compounds as possible on the regional and continental scale (*e.g.* I_{tox}-NL, I_{tox}-Europe). However, the principle of F_u-calculation and F_u-aggregation proposed in the present report is not only applicable on a continental or regional scale but also on a local scale. It is proposed to make use of the higher accuracy and spatial differentiation of measured concentrations on a local scale. Measured concentrations on a local scale allow for local F_u-assessment (per compound, or F_uTOTAL), which can be used for mapping of the potential risks of (combinations of) compounds in a certain area.

In summary, the method of F_u-assessment and F_u-aggregation is applicable for all three scales. Only F_uTOTAL on a continental or regional scale is called I_{tox}. The choice between measured and calculated input concentrations depends on the scale (*i.e.* the goal) for which the F_u-method is used. This is illustrated in the scheme below, where the current study focussed on the right part of this scheme.

	local	**regional or global**
F_u-calculation of the separate compounds	**outcome:** spatially differentiated F_u-values for one single compound **application:** - F_u-mapping per compound - indicating problem areas - compound-specific local policy *Spatial differentiation of F_u-values is only possible with reliable and differentiated concentrations: use MEASURED concentrations as input values*	**outcome:** one single regional F_u-value per compound **application** input for further regional or global aggregation of F_u-values *The regional or global F_u is based on one single regional or global, "average" concentration: use CALCULATED concentrations as input values*
F_u-aggregation of multiple compounds	**outcome:** spatially differentiated $F_{u\text{TOTAL}}$-values **application:** - F_u-mapping of multiple compounds - indicating problem areas - comparison to previous maps *In principle only possible for compounds which are monitored locally*	**outcome:** one regional or global F_u-value for all compounds together: I_{tox} **application:** (see 1.4.) - EPPI–D (regional) - TARGETS - GEO (global) *In principle only possible for compounds with known production volumes*

5.2. Potency of the F_u-method as a basis for an environmental quality indicator

In this paragraph, the method of F_u-assessment and F_u-aggregation is compared to the list of characteristics which guarantee the quality of a policy indicator (see 2.3.). The discussion described here is based on the results of chapter 4 and on the outcome of a RIVM expert workshop (Hamers 1995a; Hamers, 1995b).

5.2.1. Scientific quality

The *goal and necessity* of an environmental quality indicator has been argued in chapter 1.

The additional value of the F_u-method to relate concentrations of toxic compounds to an overall environmental quality rather than to an overall environmental pressure is also recognized by the participants of the workshop (Hamers, 1995b).

The *scientific justifiability* of the method of F_u-assessment and especially of F_u-aggregation was mainly based on the accepted principles in single species combination toxicology, *i.e.* concentration addition and effect addition. Concentration addition is applied for hydrophobic, inert compounds with a simple and similar narcotic mode of action, whereas effect addition is applied for all other compounds showing some kind of specific mode of action. Classification of compounds as narcotics is based on a method developed by Verhaar *et al.* (1992). It should be realized that this classification system is based completely on the molecular structure of the compounds. In some cases, this classification is insufficient to distinguish compounds with different toxicities. For some compounds (*e.g.* PAHs) differences in toxicity have been found, which were not expected based on similar QSARs determined by this classification (Crommentuijn (RIVM), personal communication).

Within the principles of concentration and effect addition, F_u's were aggregated in two different ways (3.2):

1 ○ standardize the NOEC frequency distributions of the narcotic compounds into HU;
○ calculate an "average" NOEC frequency distribution in terms of HU;
○ standardize the estimated field concentrations of the narcotic compounds into HU;
○ determine the F_uNARCOTIC of the sum of all standardized field concentrations from the "average" distribution function (concentration addition);
○ multiply the F_pNARCOTIC to the F_p's of all other compounds and determine F_pTOTAL and its complement F_uTOTAL.

2 ○ perform a multivariate non-linear regression analysis on the NOECs of all compounds as illustrated in 4.4, with separate parameter estimates for a and b in case of effect addition and separate and shared parameter estimates for a and b respectively in case of concentration addition;
○ determine F_uTOTAL for the combination of estimated field concentrations.

From the test calculation it was concluded that the second method is preferred, because

- it is simpler: in fact F_u-assessment and F_u-aggregation are performed in one single step, which does not require log-transformation, standardization into log(HU), nor conversion of F_u's into F_p's and *vice versa*;
- it improves the transparency of I_{tox} (see 5.2.4.);
- it avoids standardization of NOECs and field concentrations into HU;
- predictive uncertainty of F_u can be calculated.

With respect to this method, it should be remarked that applying non-linear least-squares for the regression seems to imply a model which allows for normally distributed deviations from F_uNARCOTICS. This is obviously not correct since F_u varies from 0 to 1, and so do the data. It would be more correct to assume β-distributed errors.

The method of F_u-aggregation was criticized for the fact that in case of effect addition a tolerance correlation of $r=0$ is assumed. As argued in 3.2.2., this assumption was adopted for sake of simplicity. Vaal *et al.* (1995) showed that this assumption holds when effect addition is applied for compounds with different specific modes of action, whereas $r>0$ when effect addition is applied for compounds with the same specific mode of action. *E.g.*, when parathion and malathion (same mode of action) are combined, daphnids and amphibians will be very sensitive and insensitive, respectively, to both compounds. When malathion and PCP (different modes of action) are combined, daphnids will be more sensitive to malathion than amphibians, whereas sensitivities for PCP are the other way around.

The main reason to base I_{tox} on the NOEC frequency distribution function, was that the Dutch chemical management policy also makes use of this function. However, it is disputable whether the fraction of species for which the NOEC (determined under laboratory conditions) is exceeded under field conditions is *relevant input information* to be aggregated to indicate the environmental quality.

The assumptions which are necessary to calculate a field concentration, introduce uncertainty into the indicator value, which undermines its *accuracy*. This uncertainty is not typical for I_{tox}: all indicators mentioned in chapter 1 are submitted to uncertainty due to a lack of input data which does not allow for a reliable concentration assessment.

Another source of uncertainty is introduced by application of the NOEC frequency distributions. Generally, F_u's will be determined at the (left) tail of the distribution function, *i.e.* the concentration range where the course of the function is most unreliable. At present, a method is under development to calculate the range of uncertainty around the frequency distribution (De Zwart (RIVM), personal communication). This method is based on the normal frequency distribution, because the appropriate statistics for the logistic distribution function are not at hand.

A final source of uncertainty is introduced by the assumption that $r=0$, in case of effect addition. This was discussed above.

Considering the want and the goal of I_{tox} (see chapter 1), some uncertainty is allowed in this early stage of development. Unfortunately, the sources of uncertainty in the I_{tox}-estimation can only be listed whereas they have not been quantified, yet. The uncertainty should be taken into account for further application of the indicator, whereas in future I_{tox}-calculations special methods need to be developed to minimize and quantify the uncertainty.

Reproducability of I_{tox}-calculation is dependent on a precise and consistent registering of all assumptions and methods selected for concentration assessment, F_u-assessment and F_u-aggregation. Therefore, quality assurance within modelling will get full attention.

5.2.2. Dynamics

The sensitivity of I_{tox} in time can not be deduced from the test calculation, due to a lack of input emissions or measured concentrations. Calculated concentrations of the 5 selected compounds were based on emission data from 1984 to 1987 (Table 4.1) whereas the Integrated Criteria Documents which provided measured concentrations from 1979 to 1988 (Table 4.3). Since no annual data were available, F_{uTOTAL}'s were calculated as the potential "average" toxic impact on the environmental quality during the 80's.

Some remarks can be made on the subject of I_{tox}-sensitivity in general:

- For both methods of F_u-aggregation, the considerable differences between measured and calculated field concentrations (Table 4.5) were reflected by similar differences in F_{uTOTAL}-values (Table 4.9).
- When more (than 5) compounds are processed into I_{tox}, the indicator is expected to become less sensitive for changes in the contribution of a single compound. F_u-aggregation is based on all compounds taken into account, which makes the final value less dependent on the contribution of a single compound. After all, this is not a drawback of I_{tox}, since in general the actual environmental quality will not improve either when one single compound is no longer emitted.
- Although emission reductions of a single toxic compound will probably not lead to a dramatic decline of I_{tox}, it is expected that a general emission reduction of many toxic compounds together (*e.g.* as achieved by the environmental policy in the last decade) will properly be reflected by I_{tox}.

5.2.3. Political relevance

As argued in 5.1.2., the information was not compressed to a *maximum level of aggregation*. To avoid loss of information and expressiveness of the indicator, separate I_{tox}-values were proposed for separate environmental compartments.

In Dutch chemical management policy, *target values* of $F_ui=5\%$ are set as maximum for each individual compound i. However, up to present no target value was defined for the combination effect of all compounds together. To understand the meaning of F_uTOTAL, definition of a target value for all compounds together is absolutely necessary.

In principle, I_{tox} is suited to *evaluate previous, present and future policy scenarios*. The test calculation of chapter 4 gives the impression that I_{tox} is rather sensitive to changes in total concentrations of toxic compounds (see 5.2.2.). On the other hand, I_{tox}-calculation is based on many assumptions introducing a lot of uncertainty. As stated above, special methods need to be developed to minimize and quantify the uncertainty in future I_{tox}-calculations. At present, I_{tox} is very well suited to provide a general impression of the impact of toxic compounds on the environmental quality, but its outcome is too uncertain (yet) to evaluate several policy alternatives in order to pursue a specific policy for a specific compound. In retrospective policy evaluation activities, the F_u-method can probably soon be used, by evaluating the spatially differentiated F_u-maps (see 5.1.4) which are more reliable since they are based on measured concentrations.

I_{tox} is a dimensionless figure. This is regarded as an advantage to *compare and integrate* the indicator with other (environmental) policy indicators, perhaps after standardizing the I_{tox}-value with regards to its target value.

5.2.4. Recognizability and clarity

I_{tox} should not be interpreted as the fraction of species in the field which is unprotected, but as the fraction of species tested under laboratory conditions for which the field concentration exceeds the laboratory NOEC. This definition is too complex to interpret directly. Therefore, I_{tox} should be regarded as an indicator-value within the range 0-1, which is based on laboratory toxicity data. Thus, the *interpretation* of an I_{tox}-calculation is facilitated considerable: $I_{tox}=0$ means a minimum level of potential risk of toxic compounds to the environment and $I_{tox}=1$ means a maximum level of potential risk of toxic compounds to the environment, which is not the same as a maximum level of toxification.

The methods of concentration-assessment (USES) and F_u-assessment (ETX) are *accessible instruments*. Both methods of F_u-aggregation (the Hazard Unit method and the multivariate regression analysis (3.2.)) may easily be programmed as an extension of USES or ETX. From this definition study it is not clear whether the necessary input data are easy accessible, because the test calculation was performed with very well-known compounds. As argued in 3.1., it was decided that a minimum number of only 2 NOECs is required to estimate a logistic frequency distribution of the log(NOEC)s. The minimum set of data needed by USES to estimate field concentrations is the emission quantity split up to the separate compartments together with only three physico-chemical characteristics, *i.e.* vapor pressure, K_{OW}, water solubility. When no emission quantities are known, field concentrations have to be estimated from production volumes. In that case, additional data are needed on the main category, the industrial category, the use category, and the relevant life-cycle steps.

I_{tox} is a very *transparent* indicator, in the sense that the final I_{tox}-value can be analyzed into the separate, contributions of the NOEC frequency distributions of the individual compounds, together with their specific field concentration estimate. Indicator transparency is improved when I_{tox} is calculated by multivariate non-linear regression because transformation of concentrations into HU masks the contribution of the different narcotic compounds to the F_uNARCOTIC.

5.3. Outcome of the test calculation

Comparison of Table 4.2 and 4.3 shows that measured concentrations are generally higher than calculated concentrations. For the compartments surface water and natural soil and the 5 compounds evaluated, the underestimation by concentration calculation remains within 1 order of magnitude, except for HCB in surface water. The underestimation may be explained by imported loads of toxic compounds, which were not taken into account in the calculations.

Based on the classification of Verhaar *et al.* (1992), GHCH and HCB were classified as narcotics. For GHCH, this classification is arguable: the use of GHCH as an insecticide suggests a more specific mode of action than the minimum toxicity which is based on its narcotic action.

Table 4.9 shows not only the aggregated F_uTOTAL's, but also the $\Sigma(PEC/HC_5)$, which is set equal to the $\Sigma(PEC/PNEC)$. As argued in 1.1.2., this indicator can be deduced by dividing the present EPPI–D by the total environmental volume. Summation of PEC/PNEC-ratios follows actually the principle of concentration addition, *i.e.* concentrations of all compounds are summed after standardization to their compound-specific MPC (HC_5).

For the surface water compartment, $PEC/PNEC = 0.26 < 1$. This means that the fraction of the total environmental volume which is filled up to the PNEC is smaller than 1.0. This is confirmed by the F_uTOTAL-value of 0.021 (or 0.0367), which is smaller than the F_u associated with the PNEC, *i.e.* 5%. For the natural soil, $PEC/PNEC = 1.889$ which is in line with the F_uTOTAL-value of 0.0882 which is larger than 5%. So far, the indicator-values of the present EPPI–D and I_{tox} do not contradict.

6. CONCLUSIONS

Based on the (outcome of) the test calculation and the discussion the following conclusions are drawn with regards to concentration-assessment, F_u-assessment, F_u-aggregation and I_{tox}:

- Development of an environmental quality indicator which is based on the frequency distribution function of laboratory NOECs (*i.e.* the same principle as used for MPC-estimation) is very well possible. The basic information for each individual compound is expressed in terms of F_u's, which is defined as the fraction of species tested in laboratory experiments for which the estimated field concentration exceeds the laboratory NOEC. The basic information (F_u per compound) is aggregated into a F_uTOTAL according to the general principles which are applied in single species combination toxicology, *i.e.* concentration addition and effect addition.

- Four fundamental choices have been made regarding I_{tox} which give answer to the four fundamental questions in 1.2. (arguments are given in 5.1.):
  1 I_{tox} is only based on chronic toxicity data. In case of insufficient NOECs it is proposed to convert acute toxicity data (*e.g.* L(E)C_{50}s) into NOECs;
  2 Separate indicator values are calculated for the individual environmental compartments. F_u-values of the individual compartments are not further aggregated into a F_uTOTAL-value for the total environment;
  3 I_{tox} describes the impact of toxic compounds on the environmental quality only with respect to the ecosystem, and not to human health;
  4 The regional (or even global) indicator I_{tox} will be based on calculated concentrations. When the methods for F_u-assessment and F_u-aggregation are applied on a regional scale, measured concentrations will be used.

- Two separate methods were developed to aggregate the F_u's, *i.e.* the Hazard Unit method and the multivariate non-linear regression. The latter is preferred because of its simplicity, its transparency and its ability to start from concentrations which are not log-transformed or standardized into Hazard Units.

- Based on the outcome of the test calculation, it was concluded that the indicator values calculated by means of the proposed I_{tox} and the present Environmental Policy Performance Indicator for the Theme of Dispersion (EPPI–D) do not contradict.

- The lack of available and reliable input data for a wide variety of compounds may be the major difficulty for concentration assessment and F_u-assessment. A minimum set of input data consists of the emission quantity split up to the individual environmental compartments, the vapor pressure, the K_{OW}, the water solubility and two acute or chronic toxicity data.

- Up till now, quantification of the introduced uncertainty within the procedure of I_{tox}-calculation is impossible. The following causes were distinguished for uncertainty in the I_{tox}-outcome:
  1 too few input parameters to calculate a reliable field concentration;
  2 absence of a method to calculate the range of uncertainty around the NOEC frequency distribution;
  3 in case of effect addition, the tolerance correlation is arbitrarily set to $r=0$, which represents a worst case scenario.

  It is recommended to develop additional methods to minimize and to quantify the impact of these uncertainties in future I_{tox}-calculations. Minimizing the uncertainty can be done by collecting more and better input data and by verifying the assumption that the tolerance correlation $r=0$. Quantifying the uncertainty can be done by sensitivity analysis and by development of a method to calculate the range of uncertainty around the frequency distribution function.

- Originally, I_{tox} was developed as a possible alternative for EPPI–D. By definition, an environmental policy indicator is a retrospective indicator (Adriaanse, 1993). However, there is no reason to believe that future policy scenarios can not be evaluated by means of I_{tox}. However, a pressure indicator may be better capable to evaluate future policy scenarios, because such indicator uses emission data as input parameters, whereas a quality indicator as I_{tox} makes use of calculated concentrations, which are often based on the emission data. In other words, another step of uncertainty is introduced when a quality indicator is used prospectively.

- I_{tox} should be regarded as a state indicator. It indicates the impact of toxic compounds on the environmental quality.

- Evaluation of I_{tox} with regards to the characteristics of a good (environmental policy performance) indicator, is shown in the scheme below. Based on this evaluation, it was concluded that I_{tox} meets most characteristics to a satisfactory level. The only exception is the inaccuracy of the indicator-value, which is due to the many sources of uncertainty mentioned above. Future efforts for I_{tox}-development should focus on quantifying and minimizing the uncertainty of I_{tox}.

Characteristic	I_{tox}[1]
Scientific quality	
consensus on the goal and necessity	+
scientifically justifiable way to aggregate complex information	++
relevance of the input information	±
accuracy of the indicator value	−
reproducability of the indicator calculation	+
Dynamics	±[2]
Political relevance	
maximum level of aggregation	±[3]
possibility to define target values	+
possibility to evaluate previous and present policy	+
possibility to evaluate future policy scenarios	±
possibility to compare to other indicators	+
Recognizability and clarity	
ease of interpretation	+[4]
ease of application	+
transparency	++

1: This column indicates to what extent I_{tox} meets the characteristics of a good indicator, where −− means "I_{tox} does not meet this characteristic at all" and ++ means "I_{tox} meets this characteristic very well".

2: In principle, I_{tox} aggregates the F_u's of as many toxic compounds as possible. In practice, it is proposed to start with the list of compounds given in Appendix *IV*. In that case, I_{tox} is only expected to be sensitive to a general emission reduction of many toxic compounds together, but not to an emission reduction of a particular, single toxic compound (see 5.2.2.).

3: It was consciously decided to aggregate I_{tox} to a sub-maximum level (see 5.2.1.).

4: I_{tox} should not be interpreted as the fraction of species in the field which is unprotected, but as an indicator-value (0-1) for the potential risk of toxic compounds for ecosystems, which is based on laboratory toxicity data (see 5.2.4.). Risk assessment is based on F_u's in conformity with the starting points of the present chemicals management policy in The Netherlands. The actual meaning of exceeding the MPC (*i.e.* $F_u \geq 0.05$) in terms of effects on ecosystems is studied within the framework of RIVM-project 607506 (Effect Assessment Milieu II) and is not under discussion in the present definition report.

7. PLAN OF APPROACH

The calculated I_{tox}-1996 is planned to be reported in the framework of the project Mapping Ecotoxicological Effects of Substances. For this purpose, a plan of approach is proposed in this chapter. This plan consists of 4 steps, *i.e.*

1 selection of compounds;
2 assessment of the environmental concentrations;
3 assessment of the NOEC frequency distributions;
4 F_u-aggregation.

All 4 steps (sometimes divided into sub-steps) are described below in separate paragraphs.

7.1. Selection of compounds

Theoretically, I_{tox} is able to aggregate toxicity information of all compounds released to the environment. Nevertheless, it is proposed to start I_{tox}-calculation for compounds for which many data are available, such as the priority substances (Janus *et al.*, 1994). For most of these compounds, the minimum set of input data is available to assess the compartment-specific field concentrations (see 7.2) and the NOEC distributions (see 7.3). For 5 selected priority substances, I_{tox}-calculation proved to be very well possible (Table 4.9).

Step 1:

Increase the set of 5 compounds (chapter 4) to the list of substances which is proposed by Slooff (1994) to be reported in the Environmental Outlooks 1997 (Appendix *VI*).

The list of compounds from Appendix *VI* contains also carcinogenic and radio-active substances. As stated in chapter 1, ecological risk assessment is based on toxicological endpoints as reproduction, growth, survival or photosynthesis and not on DNA-changes. Carcinogenic and radio-active compounds are reported in the Environmental Outlooks 1997 for the sake of human risk assessment and therefore they can be ignored in the calculation of I_{tox}-1996, since it was decided in 5.1.3. that I_{tox} indicates the impact of toxic compounds on the environmental quality only with respect to the ecosystem and not to human health.

7.2. Concentration assessment

7.2.1. Calculated concentrations for the regional scale

As discussed in 5.1.4., it was decided that I_{tox} is a general regional indicator, which is based on an "average" concentration for each environmental compartment, which is calculated by means of USES. Concentration calculation is either a one-step or a two-step procedure:

1. Concentrations are directly calculated from emission data (as done in chapter 4). The minimum set of input data consists of
    - emission quantities to the individual environmental compartments;
    - vapor pressure;
    - K_{OW};
    - water solubility.
2. Concentrations are calculated from emission quantities which are calculated from the production volume. The minimum set of input data consists of
    - production volume;
    - vapor pressure;
    - K_{OW};
    - water solubility;
    - main category;
    - industrial category;
    - use category;
    - relevant life-cycle steps.

When concentrations of heavy metals are calculated by USES, additional input data on the K_p(air-water), K_p(solids-water;soil), K_p(solids-water;sediment) and K_p(solids-water;suspended matter) are required in both procedures.

For the test calculation of chapter 4, the one step procedure was followed. However, the emission input data used to calculate the field concentrations dated from different years. The F_uTOTAL must be considered as an average for the mid-1980's. Obviously, this is impossible when I_{tox} is planned to be reported yearly or two-yearly. It is proposed to:

<u>Step 2:</u>

a Collect as many input data as possible to calculate the field concentration for each individual environmental compartment, preferably by the one-step method.

b Make sure that the input data "emission quantity" and/or "production volume" refer to 1996 and at least one reference year, *e.g.* 1986.

In future I_{tox}-calculations, an additional fundamental choice should be made with respect to concentrations calculation: *Should I_{tox} account for foreign emission quantities and/or production volumes?* In the test calculation, the calculated concentrations were mainly based on national emissions only. This was used as an argument to explain the difference between measured and calculated field concentrations. The answer to this fundamental question depends on the goal of an I_{tox}-calculation:

YES, if I_{tox} should provide the most realistic indication of the impact of toxic compounds on the environmental quality;

NO, if I_{tox} is used as a policy indicator which is supposed to indicate the efficacy of the Dutch environmental policy to reduce the impact of toxic compounds on the environmental quality.

This leads to the following step:

Step 2:

c **Define the goal of the I_{tox}-calculation to decide whether foreign emissions or production volumes should be taken into account or not.**

The input data should be obtained from several sources, such as (public) literature and consultation of experts from the Laboratory of Waste Materials and Emissions, RIVM-LAE).

7.2.2. Measured concentrations for the local scale

I_{tox} is an indicator for the regional scale, based on calculated concentrations, whereas the method of F_u-assessment and F_u-aggregation may as well be applied on a local scale. In 5.1.4., it was decided that local F_u's and F_uTOTAL's should be based on measured concentrations, which are spatially differentiated. Local measured concentrations should be obtained from a regional monitoring network, with a regular sample grid.

Local measured concentrations are used for mapping of F_uTOTAL and compound-specific F_u's in stead of I_{tox}-calculation. Therefore, the next step is not numbered in this plan of approach, although it is essential for *mapping* of F_u-values:

Collect measured field concentrations of the selected compounds for each individual environmental compartment in 1996 and at least one reference year, *e.g.* 1986.

Measured field concentrations, determined in monitoring programs should be obtained from reports and experts from the Laboratory of Soil and Groundwater Research (RIVM-LBG) and the Laboratory of Water and Drinking-water Research (LWD).

7.3. Assessment of the NOEC frequency distributions

For each compound selected, the NOEC frequency distribution function should be determined at least for aquatic species. Preferably, the functions for soil and sediment should be based on actual laboratory NOECs for terrestrial and sedimental species, respectively. In case of a lack of soil and sediment NOECs, the aquatic NOEC frequency distribution function may be converted by making use of the equilibrium partitioning method as illustrated for the sediment compartment in chapter 4.

Not only direct NOECs can be used to determine the distribution function. Secondary poisoning can be processed into the function by converting indirect NOECs into the corresponding concentration in the relevant environmental compartment.

A minimum of 2 NOECs is required to calculate a frequency distribution by means of the ETX-program. If less than 2 NOECs are available, acute $L(E)C_{50}$s can be converted into NOECs by means of the conversion factors used for MPC-derivation in case less than 4 NOECs are available (Appendix *III*).

As discussed in 5.2.1., the method of multivariate, non-linear regression is preferred to the determination of compound-specific NOEC frequency distributions by means of ETX. Based on the minimum number of NOECs required for ETX-calculation, the minimum number of NOECs is also set to four for regression analysis on a single compound. This means

- in case of *effect addition*, for each individual compound a minimum number of NOEC of 2 NOECs is required (each compound has its own distribution function with specific parameter estimates a and b).;
- in case of *concentration addition*, for a group consisting of n narcotic compounds a minimum number of n+1 NOECs is required (each compound has its specific location parameter estimate a, but all n compounds share the same scale parameter estimate b (see Figure 4.2).

The following procedure is proposed:

Step 3:

a collect as many direct and indirect laboratory toxicity data of the selected compounds (Appendix *VI*) for the water, soil and sediment compartment;

b convert the indirect toxicity data into direct toxicity data as illustrated in 4.3. The following set of input data is required:
  - **the critical pathway;**
  - **the caloric conversion factor;**
  - **the bioconcentration factor;**

c select the narcotic compounds by means of the method of Verhaar *et al.*, 1992;

d when step 3a and 3b provide a set of sufficient NOECs (*i.e.* direct NOECs and converted indirect NOECs), the method of multivariate non-linear regression is

executed (as illustrated in chapter 4);

e when step 3a and 3b provide insufficient NOECs, acute L(E)C_{50}s can be divided by a factor 10 and be added to the data set;

f when step 3a, 3b and 3e provide sufficient NOECs, the method of multivariate non-linear regression is executed;

g when step 3a, 3b and 3e provide insufficient NOECs for one of the environmental compartments distinguished, the compound should not be taken into consideration when calculating I_{tox} for that specific compartment. However, when sufficient data are available to calculate an aquatic distribution function but insufficient data for a terrestrial or sedimental distribution function, the aquatic frequency distribution is converted by means of k_p-factors into a terrestrial or sedimental function.

NOECs and other laboratory toxicity data should be obtained mainly from reports and public files at the Toxicology Advisory Centre (RIVM-ACT). For all relevant metals and for some pesticides, frequency distributions of aquatic NOECs are available (Crommentuijn (RIVM), personal communication).

7.4. F_u-aggregation

Finally, the F_uTOTAL is determined, based on the estimated concentrations and NOEC frequency distributions, by

$$F_{u_{TOTAL}} = 1 - \frac{1}{[1 + (\sum_j \frac{C_j}{a_j})^{b_{narcotics}}] \cdot \prod_i [1 + (\frac{C_i}{a_i})^{b_i}]}$$

where (j) is an individual narcotic compound with a specific field concentration (c_j), a specific log(HC_{50}) (a_j) and a common slope parameter ($b_{narcotics}$) whereas (i) is an individual compound with a specific mode of action and a specific field concentration (c_i), a specific log(HC_{50}) (a_i) and a specific b_i.

I_{tox} is then defined as F_uTOTAL based on calculated field concentrations on a regional scale. This leads to the final step of this plan of approach to calculate I_{tox}:

Step 4:

Determine I_{tox} (F_uTOTAL of the selected compounds (see 7.1)) by filling out the equation above. Use the following input parameters:

1 calculated field concentrations from 7.2;

2 parameter estimates a and b of the NOEC frequency distribution from 7.3.

On a local scale, F_uTOTAL for a sampling point area is based on the measured concentrations. Finally, the F_uTOTAL-values for the various sample points can be mapped, in order to indicate problem areas and show the spatial shift in time of the toxic impact on the environment. Again, the subjoined step is not numbered in this plan of approach, because it leads to F_u-mapping rather than to the goal of this plan of approach (*i.e.* I_{tox}-determination):

Map the local F_uTOTAL's of the selected compounds (see 7.1)) which are calculated by filling out the equation above. Use the following input parameters:

1 measured local field concentrations from monitoring networks (see 7.2);

2 parameter estimates a and b of the NOEC frequency distribution from 7.3.

GLOSSARY

AChE: Acetyl-cholinesterase.

BCF: Bioconcentration factor.

ccf: Caloric conversion factor.

Cd: Cadmium - a compound selected for the test calculation of I_{tox} in chapter 4.

Cu: Copper - a compound selected for the test calculation of I_{tox} in chapter 4.

Dispersion: The issue defined in the Dutch National Environmental Policy Plan (NEPP, 1989) which concerns the pollution with and the adverse environmental effects of environmentally hazardous substances and of genetically modified organisms on man, flora and fauna.

DT_x: Disappearance Time x - the time it takes before a fraction x of an initial quantity of a compound has disappeared, either by biotic or abiotic processes.

EC_x: Effect Concentration x% - The concentration which affects x% of the test population in a single species laboratory experiment.

ED_x: Effect Dose x% - The dose which affects x% of the test population in a single species laboratory experiment.

EPPI: Environmental Policy Performance Indicator - An indicator which illustrates the impact of policy measures on the state of affairs of the environmental themes. For each theme a specific EPPI is defined. Each indicator contains theme-specific condensed information on the pressure (*i.e.* the stress factor which causes the environmental problem) and the possible effects of this pressure in a certain area (*e.g.* The Netherlands).

EPPI-D: Environmental Policy Performance Indicator for the theme of Dispersion.

ETX: Ecotoxicological Extrapolation Program - A program to calculate confidence limits for Hazardous Concentrations based on small samples of toxicity data.

F_{pA}: Fraction protected A - Fraction of all species for which the NOEC is not exceeded by compound A in the field situation. F_{pA} is the complement of F_{uA}, *i.e.* $F_{pA} = 1 - F_{uA}$.

F_{uA}: Fraction unprotected A - Fraction of all species for which the NOEC is exceeded by compound A in the field situation.

GEO: Global Environmental Outlook.

GHCH: γ-hexachlorohexane (lindane) - a compound selected for the test calculation of I_{tox} in chapter 4.

HCB: Hexachlorobenzene - a compound selected for the test calculation of I_{tox} in chapter 4.

HC_x: Hazardous Concentration x% - The concentration at which the NOEC is exceeded for x% of all species tested under laboratory conditions.

HU: Hazard Unit - Arbitrary dimensionless unit to express the hazard of a compound at a given concentration for more than one species. A concentration is standardized into HU's by dividing it by its compound-specific HC_{50}. In other words, 1 HU is defined as the standardized equivalent of the HC_{50}.

Independent Action: A combination effect of more than one compound on a single species. Independent Action is characterized by the different modes of action and no interaction between te compounds. Effect addition is restricted to compounds showing Independent Action.

I_{tox}: Indicator Effects Toxic Substances - The total fraction of species tested under laboratory conditions for which the NOEC is exceeded as a result of the simultaneous presence of many chemicals under field conditions.

K_pX-Y: Partitioning coefficient - Concentration of a compound in environmental compartment X divided by its concentration in compartment Y.

LC_x: Lethal Concentration x% - The concentration which causes mortality for x% of the test population in a single species laboratory experiment.

LD_x: Lethal Dose x% - The dose which causes mortality for x% of the test population in a single species laboratory experiment.

MPC_i: Maximum Permissible Concentration of compound i in a defined environmental compartment.

NEPP: National Environmental Policy Plan.

NOEC: No Observed Effect Concentration, *i.e.* the highest concentration tested in a laboratory toxicity experiment which causes a response that does not differ significantly from the control response.

OP-esters: Organo-phosphorous esters.

PAHs: Polycyclic Aromatic Hydrocarbons.

PCP: Pentachlorophenol - a compound selected for the test calculation of I_{tox} in chapter 4.

PEC_i: Predicted Environmental Concentration of compound i in a defined environmental compartment.

$PNEC_i$: Predicted No Effect Concentration of compound i in a defined compartment.

PSR-chain: Causality chain consisting of three major steps that are followed by a compound in the environment, *i.e.* first it causes pressure (P) or stress on the environment, next it affects the state (S) of the environment and finally it causes a response (R).

QSAR: Quantitative Structure-Activity Relationship.

SSA: Simple Similar Action - A combination effect of more than one compound on a single species. SSA is characterized by the same mode of action and no interaction between te compounds. Concentration addition is restricted to compounds showing SSA.

Tolerance correlation: Rank correlation between the sensitivity of organisms for separately applied compounds.

TU: Toxic Unit - Arbitrary dimensionless unit to express the toxicity of a compound at a given concentration for a single species. A concentration is standardized into TU's by dividing it by its compound-specific LC_{50}. In other words, 1 TU is defined as the standardized equivalent of the LC_{50}.

USES: Uniform System for Evaluation of Substances (RIVM *et al.*, 1994).

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APPENDIX *I*: List of experts interviewed and invited to participate in I_{tox}-workshop, 19-09-1995.

DGM-SVS:	Dr. A. Sedee
RITOX:	Dr. J.L.M. Hermens
RIVM-ACT:	Dr. G.H. Crommentuijn
	Drs. J.A. Janus
	Dr. W.H. Könemann
	Dr. W.C. Mennes
	Dr. J.W. Tas
	Drs. E. van de Plassche
RIVM-CCM:	Drs. A.E.M. de Hollander
RIVM-CWM:	Prof.Dr.Ir. J. Rotmans
RIVM-ECO:	Drs. J.H. Canton
	Drs. D. de Zwart
	Dr. H.J.P. Eijsackers
	Drs. T.P. Traas
	Ir. M.A. Vaal
RIVM-LBG:	Drs. B.J.E. ten Brink
RIVM-LSO:	Dr. M.P.M. Janssen
RIVM-MBV:	Dr. W. Slooff
RIVM-TOX:	Dr.Ir. E. Buringh

APPENDIX *II*: Characteristics of the logistic and normal distribution functions

LOGISTIC:

Density function:

$$f(x) = \frac{e^{\frac{-(x-\alpha)}{\beta}}}{\beta \cdot [1 + e^{\frac{-(x-\alpha)}{\beta}}]^2}$$

Distribution function:

$$F(x) = [1 + e^{\frac{-(x-\alpha)}{\beta}}]^{-1}$$

where $\alpha = \mu$ and

$$\beta = \frac{\sigma \cdot \sqrt{3}}{\pi}$$

Median:

$$Median(x) = \alpha$$

NORMAL:

Density function:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

Distribution function:

$$F(x) = \int_{-\infty}^{x} f(t)dt$$

Median:

$$Median(x) = \mu$$

APPENDIX *III*: Proposed method to estimate the location and scale parameters (α and β) of a logistic distribution of log(NOEC)s, based on the assessment factors generally accepted for MPC derivation in the case that less than 4 NOECs are available.

In the case that less than 4 NOECs of a compound are found in the literature, Maximum Permissible Concentrations (MPCs) are derived from NOECs and/or L(E)C_{50}s by means of assessment factors (modified EPA-method). The following definition scheme is applied for MPC-derivation (Van der Plassche, 1994):

number of chronic NOECs or QSAR estimates[a]	number of acute L(E)C_{50}s or QSAR estimates		
	0	1-2	$\geq 3^b$
0	No MPC derived	MPC = $(LC_{50})_{min}/1000$	MPC = $(LC_{50})_{min}/100$
1-2	MPC = $(NOEC)_{min}/10$	**if** $(LC_{50})_{min}/100 \leq (NOEC)_{min}$ MPC = $(LC_{50})_{min}/1000$ **else** MPC = $(NOEC)_{min}/10$	**if** $(LC_{50})_{min}/10 \leq (NOEC)_{min}$ MPC = $(LC_{50})_{min}/100$ **else** MPC = $(NOEC)_{min}/10$
3^b	MPC = $(NOEC)_{min}/10$	MPC = $(NOEC)_{min}/10$	MPC = $(NOEC)_{min}/10$

a: In the case that ≥4 NOECs are available, MPC is defined as the 5th percentile of the logistic distribution of the log(NOEC)s, which is calculated by means of ETX.

b: NOECs should refer to organisms belonging to three different trophic levels.

Originally, MPC-derivation out of few toxicity data was based on three extrapolation steps. To each of these steps an assessment factor of 10 is attributed:

- From laboratory to field;
- From L(E)C_{50} to NOEC;
- From acute to chronic toxicity data.

Let us now consider the upper row of the scheme above, *i.e.* either 1-2 or more than 3 LC_{50}s are available and no NOECs. Application of the three extrapolation steps mentioned above means that in both cases an assessment factor of 1000 should be applied. Apparently, an extra assessment factor (10) is used here to correct for number of LC_{50}s available. This extra factor is not used in the first column: irrespective of the number of NOECs (1, 2 or 3) the same assessment factor of 10 is used to derive the MPC. However, proposals are made now to raise the assessment factor in case of 1-2 NOECs and no LC_{50}s to 100 (Crommentuijn (RIVM), personal communication).

Furthermore, the extrapolation scheme above shows that equal assessment factor is used in case of 1 or 2 toxicity data. For instance, in the case that only 1 NOEC is available, the same application factor is applied on this single value as on the lowest NOEC ($NOEC_{min}$) in the case that only 2 NOECs are available. It is proposed here, that a correction step should be made for the number of available toxicity data: a single NOEC should be divided by a factor 10 to determine "$NOEC_{min}$" = NOEC/10, where "$NOEC_{min}$" is the expected minimum NOEC when 2 or 3 NOECs would be available in stead of the single NOEC which is actually available. In further MPC-assessment, "$NOEC_{min}$" should be modified similarly to an actual $NOEC_{min}$ which is determined from a dataset of 2 or 3 NOECs.

Based on the discussion above, a proposal is made to adapt the extrapolation scheme above:

number of chronic NOECs or QSAR estimates[a]	number of acute $L(E)C_{50}$s or QSAR estimates		
	0	1	≥ 2[b]
0	No MPC derived	MPC = $(LC_{50})/1000$	MPC = $(LC_{50})_{min}/100$
1	MPC = (NOEC)/100	if $(LC_{50})/10 \leq$ NOEC MPC = $(LC_{50})/100$ else MPC = (NOEC)/10	if $(LC_{50})_{min}/10 \leq$ NOEC MPC = $(LC_{50})_{min}/100$ else MPC = (NOEC)/10
2-3[b]	MPC = $(NOEC)_{min}/10$	MPC = $(NOEC)_{min}/10$	MPC = $(NOEC)_{min}/10$

a: In the case that ≥4 NOECs are available, MPC is defined as the 5th percentile of the logistic distribution of the log(NOEC)s, which is calculated by means of ETX.

b: NOECs should refer to organisms belonging to three different trophic levels.

This scheme, is based on 3 extrapolation steps with corresponding assessment factors of 10:

- From $NOEC_{min}$ to MPC;
- From a single NOEC to "$NOEC_{min}$";
- From LC_{50} to NOEC.

If the log(MPC) derived by this assessment method is regarded equal to the 5th percentile of the logistic distribution or log(NOEC)s, the location parameter α and the scale parameter β of this distribution are actually defined. This is demonstrated below, where $y=\log(LC_{50})$ and $z=\log(NOEC)$.

NB: Van der Plassche (1994) describes two possible methods for including secondary poisoning in MPC-determination:

1. *calculation of two separate MPCs,* i.e. *one for the set of toxicity data on lower organisms and one for the set of toxicity data on top predators, whereafter the lowest is selected as MPC (Method I);*
2. *calculation of one single MPC for the combined set of toxicity data on both lower organisms and top predators (Method II).*

In the present study it was tried to calculate the ***total*** *fraction of species for which the laboratory NOEC is exceeded under field conditions. With respect to this fraction of organisms, no difference was made whether NOECs were exceeded for lower organisms or for top predators.* F_u*-values were determined for both groups of organisms together, and not separately. Therefore, NOEC distributions are determined for the combined set data on both lower organisms and top predators (Method II). This is in contrast with the final decision made by Van der Plassche (1994) who used method I for MPC-determination.*

*As shown in the footnotes of the schemes above, additional requirements are made considering the taxonomical groups which are represented when Method I is used for MPC-derivation (*i.e. *in the case that less than 4 NOECs are available). For Method II these requirements are not made. Since NOEC distributions in the present study are based on combined data sets of both lower organisms and top predators (Method II) it is decided that no extra requirements with respect to taxonomical groups represented should be made in the proposal described below to determine a logistic distribution of the log(NOEC)s based on the assessment factors.*

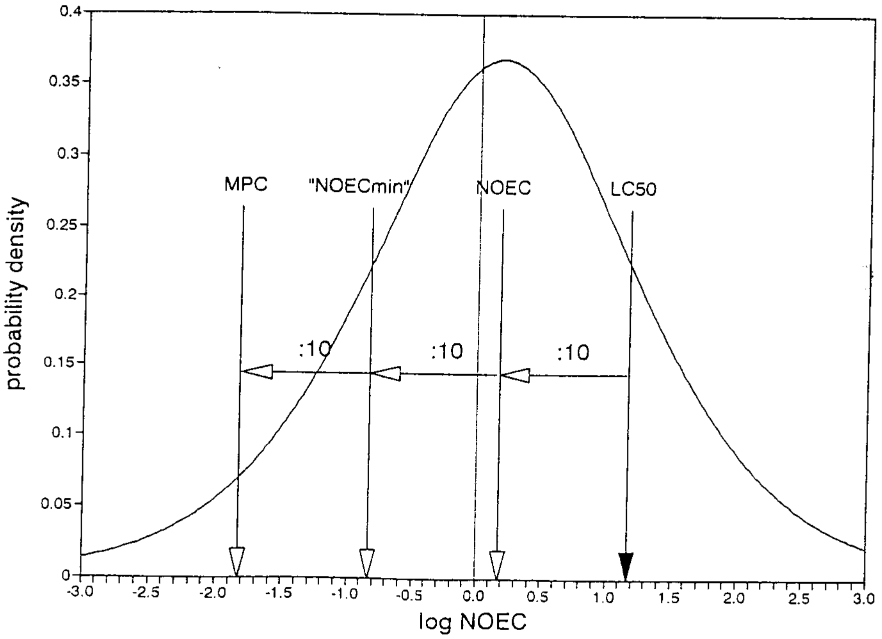
1 LC_{50} and 0 NOECs:

To recalculate the LC_{50} into a NOEC, the single LC_{50} is divided by a factor 10:
$\log(NOEC) = \log(LC_{50}/10) = y-1$.
Since y-1 is the only NOEC available, it is regarded as the median of the distribution function.
To determine $NOEC_{min}$, the median NOEC is again divided by a factor 10:
$\log(NOEC_{min}) = \log(NOEC/10) = \log((LC_{50}/10)/10) = y-2$.
To determine MPC, $NOEC_{min}$ is again divided by a factor 10:
$\log(MPC) = \log(NOEC_{min}/10) = \log((NOEC/10)/10) = \log(((LC_{50}/10)/10)/10) = y-3$.
Regarding this MPC equal to an MPC determined as the 5th percentile from a fitted frequency distribution function implies that y-3 is the 5th percentile of the frequency distribution function of the specific case. This results in two equations:

$$F_u(y-1) = 0.5 = [1 + e^{\frac{-y+1+\alpha}{\beta}}]^{-1}$$

$$F_u(y-3) = 0.05 = [1 + e^{\frac{-y+3+\alpha}{\beta}}]^{-1}$$

From these equations, it is derived that α = **y-1** and β = **2/ln(19)**. This is illustrated in the figure below.



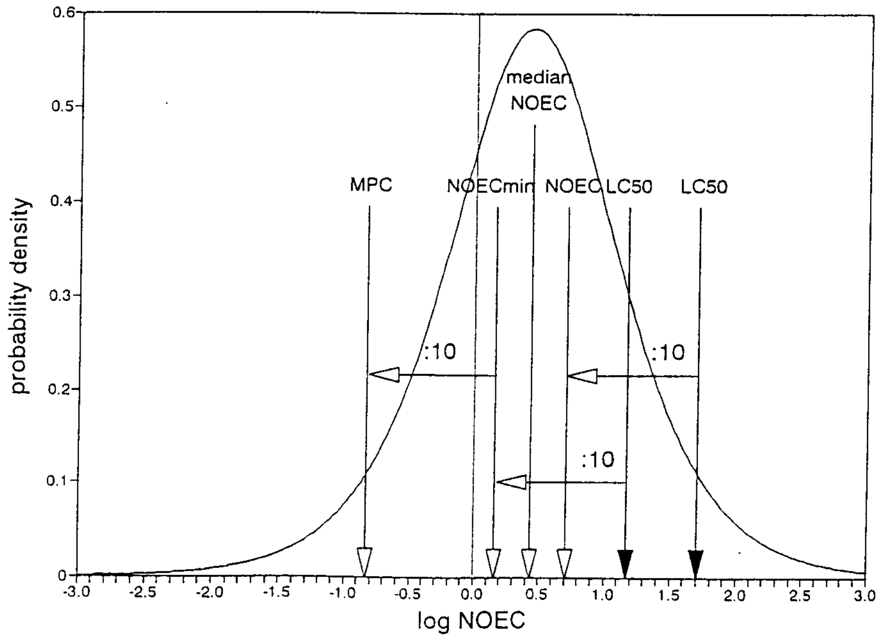
$\geq$2 LC$_{50}$s and 0 NOECs:

To recalculate the LC$_{50}$s into NOECs, the LC$_{50}$s are divided by a factor 10:
log(NOEC) = log(LC$_{50}$/10) = y-1.
Next, the average of the calculated NOECs is regarded as the median of the distribution function.
To determine NOEC$_{min}$, the LC$_{50min}$ is also divided by a factor 10:
log(NOEC$_{min}$) = log(LC$_{50min}$/10) = y$_{min}$-1.
To determine MPC, NOEC$_{min}$ is again divided by a factor 10:
log(MPC) = log(NOEC$_{min}$/10) = log((LC$_{50min}$/10)/10) = y$_{min}$-2.
Regarding this MPC equal to an MPC determined as the 5th percentile from a fitted frequency distribution function implies that y$_{min}$-2 is the 5th percentile of the frequency distribution function of the specific case. This results in two equations:

$$F_u(\bar{y}-1) = 0.5 = [1 + e^{\frac{-\bar{y} + 1 + \alpha}{\beta}}]^{-1}$$

$$F_u(y_{min}-2) = 0.05 = [1 + e^{\frac{-y_{min} + 2 + \alpha}{\beta}}]^{-1}$$

From these equations, it is derived that α = $\bar{y}$-1 and β = ($\bar{y}$-y$_{min}$+1)/ln(19). This is illustrated in the figure below.



0 LC_{50}s and 1 NOEC:

The only NOEC available is regarded as the median of the distribution function.

To determine $NOEC_{min}$, this median NOEC is divided by a factor 10:

$\log(NOEC_{min}) = \log(NOEC/10) = z\text{-}1$.

To determine MPC, $NOEC_{min}$ is again divided by a factor 10:

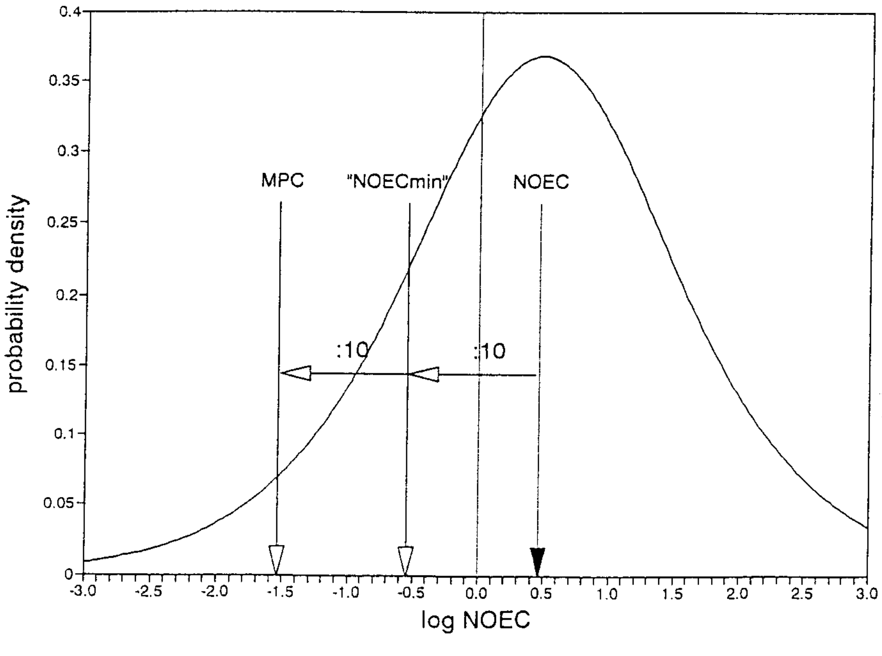
$\log(MPC) = \log(NOEC_{min}/10) = \log((NOEC/10)/10) = z\text{-}2$.

Regarding this MPC equal to an MPC determined as the 5th percentile from a fitted frequency distribution function implies that z-2 is the 5th percentile of the frequency distribution function of the specific case. This results in two equations:

$$F_u(z) = 0.5 = [1 + e^{\frac{-z + \alpha}{\beta}}]^{-1}$$

$$F_u(z-2) = 0.05 = [1 + e^{\frac{-z + 2 + \alpha}{\beta}}]^{-1}$$

From these equations, it is derived that α **= z** and β **= 2/ln(19)**. This is illustrated in the figure below.



1 LC50 and 1 NOEC with $(LC_{50}/10) \leq NOEC$:

To recalculate the LC_{50} into a NOEC, the LC_{50} is divided by a factor 10:
$\log(NOEC) = \log(LC_{50}/10) = y\text{-}1$.

Next, the average of the actual and calculated NOECs is regarded as the median of the distribution function. Since $y\text{-}1 \leq z$, $NOEC_{min}$ is determined by dividing LC_{50} by a factor 10:
$\log(NOEC_{min}) = \log(LC_{50}/10) = y\text{-}1$.

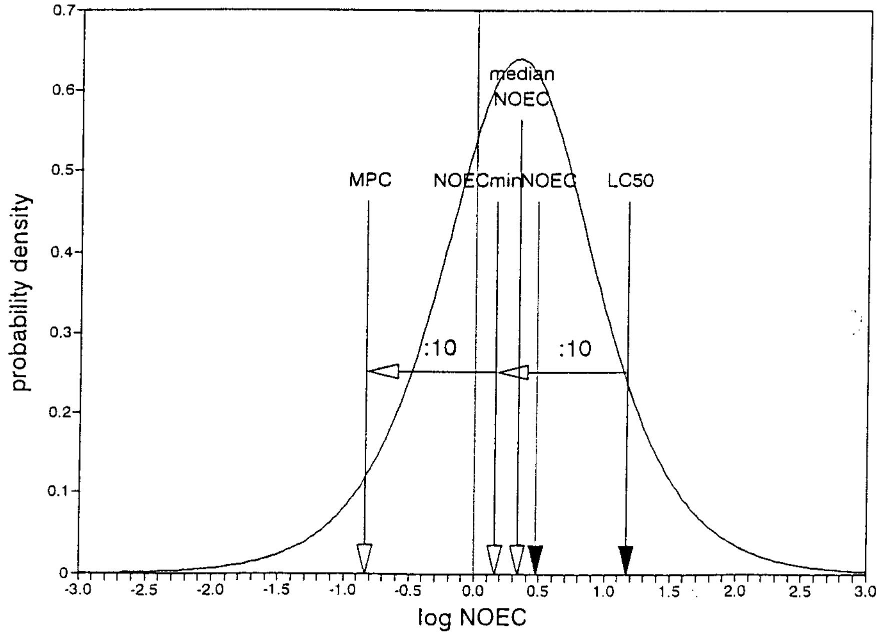
To determine MPC, $NOEC_{min}$ is again divided by a factor 10:
$\log(MPC) = \log(NOEC_{min}/10) = \log((LC_{50}/10)/10) = y\text{-}2$.

Regarding this MPC equal to an MPC determined as the 5th percentile from a fitted frequency distribution function implies that y-2 is the 5th percentile of the frequency distribution function of the specific case. This results in two equations:

$$F_u\left(\frac{y-1+z}{2}\right) = 0.5 = \left[1 + e^{\frac{-y+1-z+2\cdot\alpha}{2\cdot\beta}}\right]^{-1}$$

$$F_u(y-2) = 0.05 = \left[1 + e^{\frac{-y+2+\alpha}{\beta}}\right]^{-1}$$

From these equations, it is derived that $\alpha = (y\text{-}1+z)/2$ and $\beta = (z\text{-}y+3)/(2 \cdot \ln(19))$. This is illustrated in the figure below.



1 LC50 and 1 NOEC with NOEC$\leq$(LC$_{50}$/10):

To recalculate the LC$_{50}$ into a NOEC, the LC$_{50}$ is divided by a factor 10:
log(NOEC) = log(LC$_{50}$/10) = y-1.

Next, the average of the actual and calculated NOECs is regarded as the median of the distribution function. Since z$\leq$y-1, the single NOEC is defined as NOEC$_{min}$.

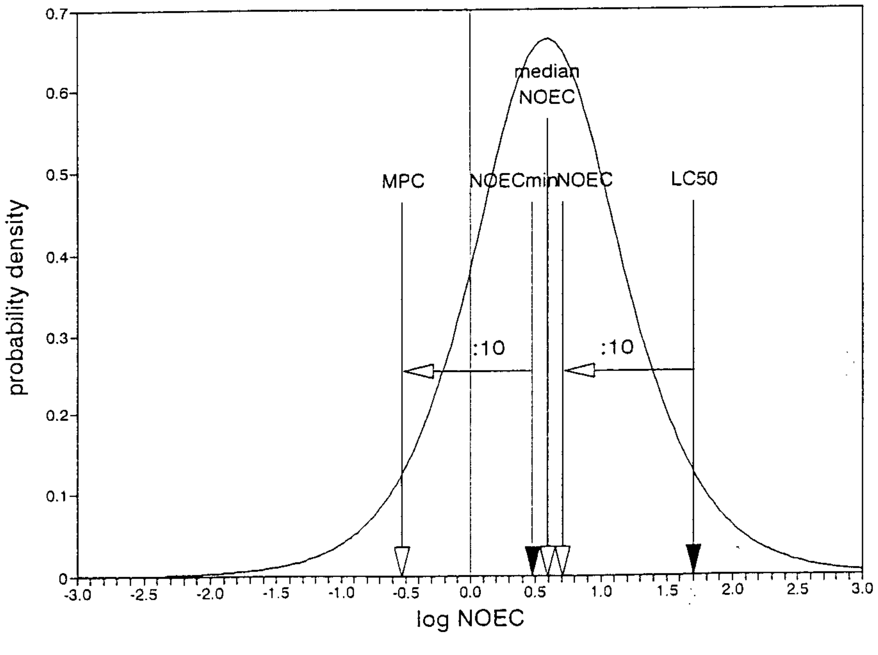
To determine MPC, NOEC$_{min}$ is divided by a factor 10:
log(MPC) = log(NOEC$_{min}$/10) = log(NOEC/10) = z-1.

Regarding this MPC equal to an MPC determined as the 5th percentile from a fitted frequency distribution function implies that z-1 is the 5th percentile of the frequency distribution function of the specific case. This results in two equations:

$$F_u\left(\frac{y-1+z}{2}\right) = 0.5 = \left[1 + e^{\frac{-y+1-z+2\cdot\alpha}{2\cdot\beta}}\right]^{-1}$$

$$F_u(z-1) = 0.05 = \left[1 + e^{\frac{-z+1+\alpha}{\beta}}\right]^{-1}$$

From these equations, it is derived that α = **(y-1+z)/2** and β = **(y-z+1)/(2 · ln(19))**. This is illustrated in the figure below.



$\geq$2 LC50s and 1 NOEC with $(LC_{50min}/10) \leq NOEC$:

To recalculate the LC_{50}s into NOECs, the LC_{50}s are divided by a factor 10:
$\log(NOEC) = \log(LC_{50}/10) = y-1$.

Next, the average of the actual and calculated NOECs is regarded as the median of the distribution function. Since $y_{min}-1 \leq z$, $NOEC_{min}$ is determined by dividing LC_{50min} by a factor 10:

$\log(NOEC_{min}) = \log(LC_{50min}/10) = y_{min}-1$.

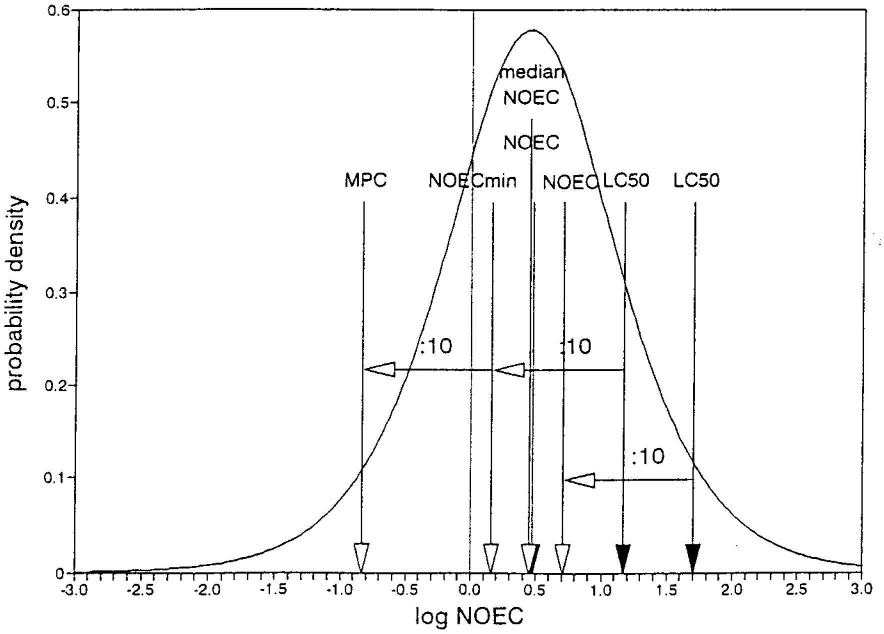
To determine MPC, $NOEC_{min}$ is again divided by a factor 10:
$\log(MPC) = \log(NOEC_{min}/10) = \log((LC_{50min}/10)/10) = y_{min}-2$.

Regarding this MPC equal to an MPC determined as the 5th percentile from a fitted frequency distribution function implies that y-2 is the 5th percentile of the frequency distribution function of the specific case. This results in two equations:

$$F_u\left(\frac{\sum_{i=1}^{n}(y_i-1) + z}{(n+1)}\right) = 0.5 = \left[1 + e^{\frac{-\sum_{i=1}^{n}(y_i) + n - z + (n+1)\cdot\alpha}{(n+1)\cdot\beta}}\right]^{-1}$$

$$F_u(y_{min}-2) = 0.05 = \left[1 + e^{\frac{-y_{min} + 2 + \alpha}{\beta}}\right]^{-1}$$

where n is number of LC_{50}s. From these equations, it is derived that **$\alpha = [\Sigma(y)+n-z]/(n+1)$** and **$\beta = (-y_{min}+2+\alpha)/(\ln(19))$**. This is illustrated in the figure below.



≥2 LC50s and 1 NOEC with NOEC≤(LC_{50min}/10):

To recalculate the LC_{50}s into NOECs, the LC_{50}s are divided by a factor 10:

log(NOEC) = log(LC_{50}/10) = y-1.

Next, the average of the actual and calculated NOECs is regarded as the median of the distribution function. Since $z \leq y_{min}-1$, the single NOEC is defined as $NOEC_{min}$.

To determine MPC, $NOEC_{min}$ is divided by a factor 10:

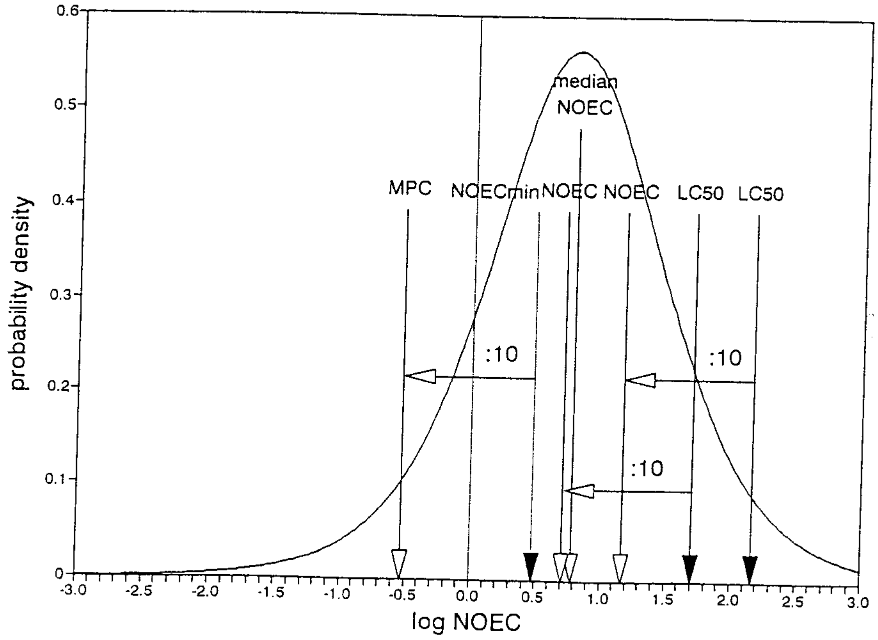
log(MPC) = log($NOEC_{min}$/10) = log(NOEC/10) = z-1.

Regarding this MPC equal to an MPC determined as the 5th percentile from a fitted frequency distribution function implies that z-1 is the 5th percentile of the frequency distribution function of the specific case. This results in two equations:

$$F_u\left(\frac{\sum_{i=1}^{n}(y_i-1)+z}{(n+1)}\right) = 0.5 = \left[1+e^{\frac{-\sum_{i=1}^{n}(y_i)+n-z+(n+1)\cdot\alpha}{(n+1)\cdot\beta}}\right]^{-1}$$

$$F_u(z-1) = 0.05 = \left[1+e^{\frac{-z+1+\alpha}{\beta}}\right]^{-1}$$

where n is number of LC_{50}s. From these equations, it is derived that α = **[Σ(y)+n-z]/(n+1)** and β = **(-z+1+α)/(ln(19))**. This is illustrated in the figure below.



≥0 LC_{50}s and 2 or 3 NOECs:

The LC_{50}s are not recalculated into NOECs. The average of only the actual NOECs is regarded as the median of the distribution function. The smallest actual NOEC-value is defined as $NOEC_{min}$.

To determine MPC, $NOEC_{min}$ is divided by a factor 10:

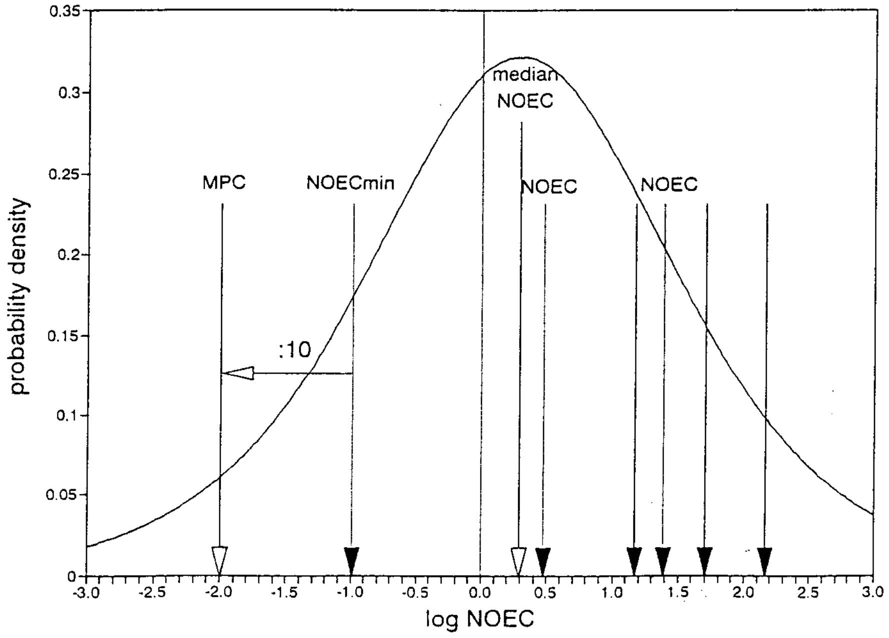
$\log(MPC) = \log(NOEC_{min}/10) = z_{min}-1$.

Regarding this MPC equal to an MPC determined as the 5th percentile from a fitted frequency distribution function implies that $z_{min}-1$ is the 5th percentile of the frequency distribution function of the specific case. This results in two equations:

$$F_u(\bar{z}) = 0.5 = [1 + e^{\frac{-\bar{z} + \alpha}{\beta}}]^{-1}$$

$$F_u(z_{min}-1) = 0.05 = [1 + e^{\frac{-z_{min} + 1 + \alpha}{\beta}}]^{-1}$$

From these equations, it is derived that $\alpha = \bar{z}$ and $\beta = (\bar{z} - z_{min} + 1)/\ln(19)$. This is illustrated in the figure below.



This whole procedure of α- and β-assessment is summarized in the table below which shows how to estimate the parameters of the NOEC frequency distribution function for each situation with less than 4 NOECs ($y=\log(LC_{50})$ and $z=\log(NOEC)$):

number of chronic NOECs or QSAR estimates[a]	number of acute L(E)C_{50}s or QSAR estimates		
	0	1	≥2
0	No values are estimated for α and β.	$\alpha = y\text{-}1$ $\beta = 2/\ln(19)$	$\alpha = \overline{y}\text{-}1$ $\beta = (\overline{y}\text{-}y_{min}+1)/\ln(19)$
1	$\alpha = z$ $\beta = 2/\ln(19)$	**if** $(LC_{50})/10 \leq NOEC$ $\alpha = (y\text{-}1+z)/2$ $\beta = (z\text{-}y+3)/(2 \cdot \ln(19))$ **else** $\alpha = (y\text{-}1+z)/2$ $\beta = (y\text{-}z+1)/(2 \cdot \ln(19))$	**if** $(LC_{50})_{min}/10 \leq NOEC$ $\alpha = [\Sigma(y)+n\text{-}z]/(n+1)$ $\beta = (\text{-}y_{min}+2+\alpha)/(\ln(19))$ **else** $\alpha = [\Sigma(y)+n\text{-}z]/(n+1)$ $\beta = (\text{-}z+1+\alpha)/(\ln(19))$
2-3	$\alpha = \overline{z}$ $\beta = (\overline{z}\text{-}z_{min}+1)/\ln(19)$	$\alpha = \overline{z}$ $\beta = (\overline{z}\text{-}z_{min}+1)/\ln(19)$	$\alpha = \overline{z}$ $\beta = (\overline{z}\text{-}z_{min}+1)/\ln(19)$

a: In the case that ≥4 NOECs are available, NOEC is defined as the 5th percentile of the logistic distribution of the log(NOEC)s, which is calculated by means of ETX.

APPENDIX *IV*: Transformation of the logistic cumulative distribution function of log(NOEC)s into a distrbution function of the linear (untransformed) NOECs.

$$F_u = [1+e^{\frac{-\log(c)+\alpha}{\beta}}]^{-1} = [1+e^{(-\log(c)+\alpha)\cdot\frac{1}{\beta}}]^{-1}$$

$$= [1+e^{(-\frac{\ln(c)}{ln10}+\alpha)\cdot\frac{1}{\beta}}]^{-1} = [1+e^{(-\ln(c)+\alpha\cdot\ln(10))\cdot\frac{1}{\beta\cdot\ln(10)}}]^{-1}$$

$$= [1+(c^{-1}\cdot 10^{\alpha})^{\frac{1}{\beta\cdot\ln(10)}}]^{-1} = [1+(c\cdot 10^{-\alpha})^{-\frac{1}{\beta\cdot\ln(10)}}]^{-1}$$

$$= \frac{1}{1+(c\cdot 10^{-\alpha})^{-\frac{1}{\beta\cdot\ln(10)}}} = \frac{(c\cdot 10^{-\alpha})^{\frac{1}{\beta\cdot\ln(10)}}}{(c\cdot 10^{-\alpha})^{\frac{1}{\beta\cdot\ln(10)}}+1} = \frac{(\frac{c}{a})^{b}}{1+(\frac{c}{a})^{b}}$$

with

$$b = \frac{1}{\beta\cdot\ln(10)}$$

and

$$a = 10^{\alpha}$$

APPENDIX *V*: Estimates for the partition coefficients of Cd and Cu.

Partition	Cd	Cu
Air-Water[1][l/l]	10^{-20}	10^{-20}
Suspended matter-Water[2][l/kg]	$10^{4.92} = 83176$	$10^{4.54} = 34674$
Sediment-Water[3][l/kg]	55451	23116
Soil Water[4][l/kg]	706	241

1 set at an extreme low value, as suggested by EU (1995);

2 Van de Plassche, 1994 (Table 3.6);

3 Based on a ratio of Kp(suspended matter-water) : Kp(sediment-water) = 1.5 (Van de Meent, personal communication);

4 Van den Hoop, in press.

APPENDIX *VI*: Proposal of compounds to be reported in Environmental Outlooks 1997 (Slooff, 1994)

Cadmium
Chromium
Copper
Mercury
Lead
Nickel
Zinc
Aluminium
Rare earth metals
Particulate Matter (PM10)
Benzo[a]pyrene
Fluoranthene
Fluorids
Dioxines
Benzene
Trichlorbenzene
Hexachlorbenzene
Pentachlorophenol
Diethylhexylphtalate
Butylphtalate
CO
NO_2
SO_2
Radon
Ozon/Volatile organics
Lindane
PCBs
Oil
Asbestos
Black smoke

"All" agricultural pesticides
non-agricultural pesticides:
  Tributyltin
  Triphenyltin

Radiation