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Environment Agency

EQSs under the Water Framework Directive: purpose, derivation and implementation

Paul Whitehouse

Environment Agency, UK

NORMAN Workshop

July 2013

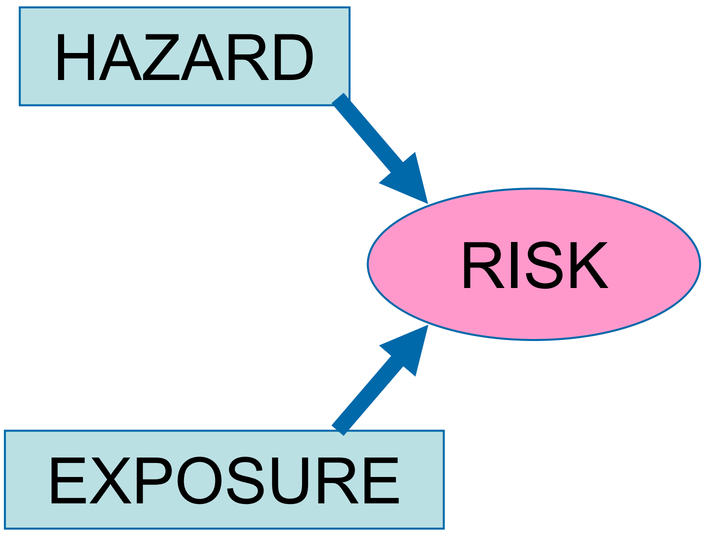
norman

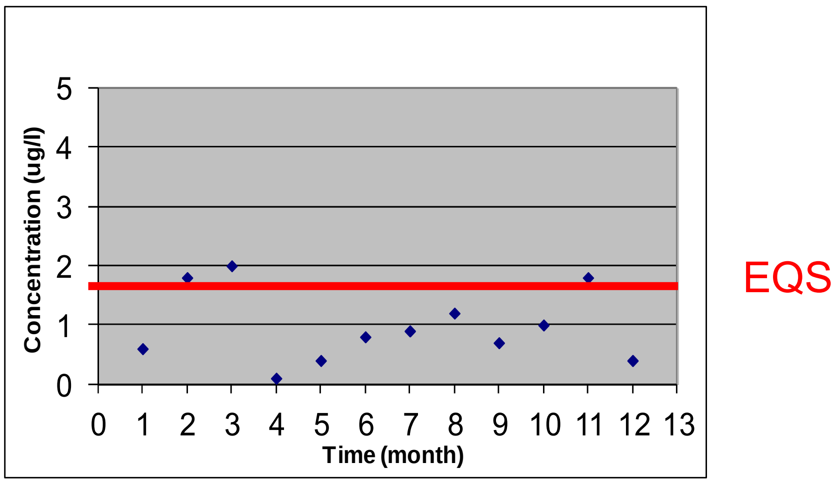
What I plan to cover ...

- Very brief introduction to what the WFD says about chemicals
- Environmental Quality Standards – how do we use them?
- How do we derive them?
- Using EQSs to classify waterbodies

What are EQSs?

- Environmental Quality Standards
- Thresholds below which we do not expect adverse effects to occur
  - Hazard-based
  - Analyse environmental samples to assess compliance (= risk)





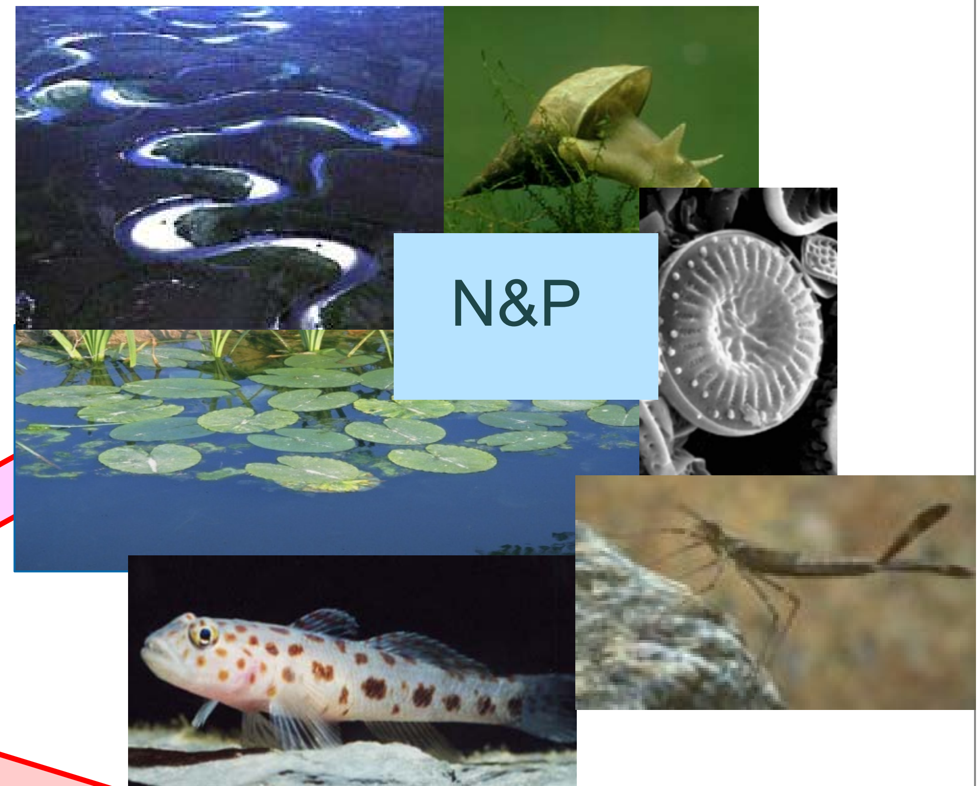
- EQS expressed as
  - a numerical value (concentration)
  - period over which the standard applies (e.g. a year) and
  - compliance statistic (e.g. mean)

Water Framework Directive: Status Objectives

Surface water status assessed using:

Chemical status

Ecological status



PRIORITY SUBSTANCES

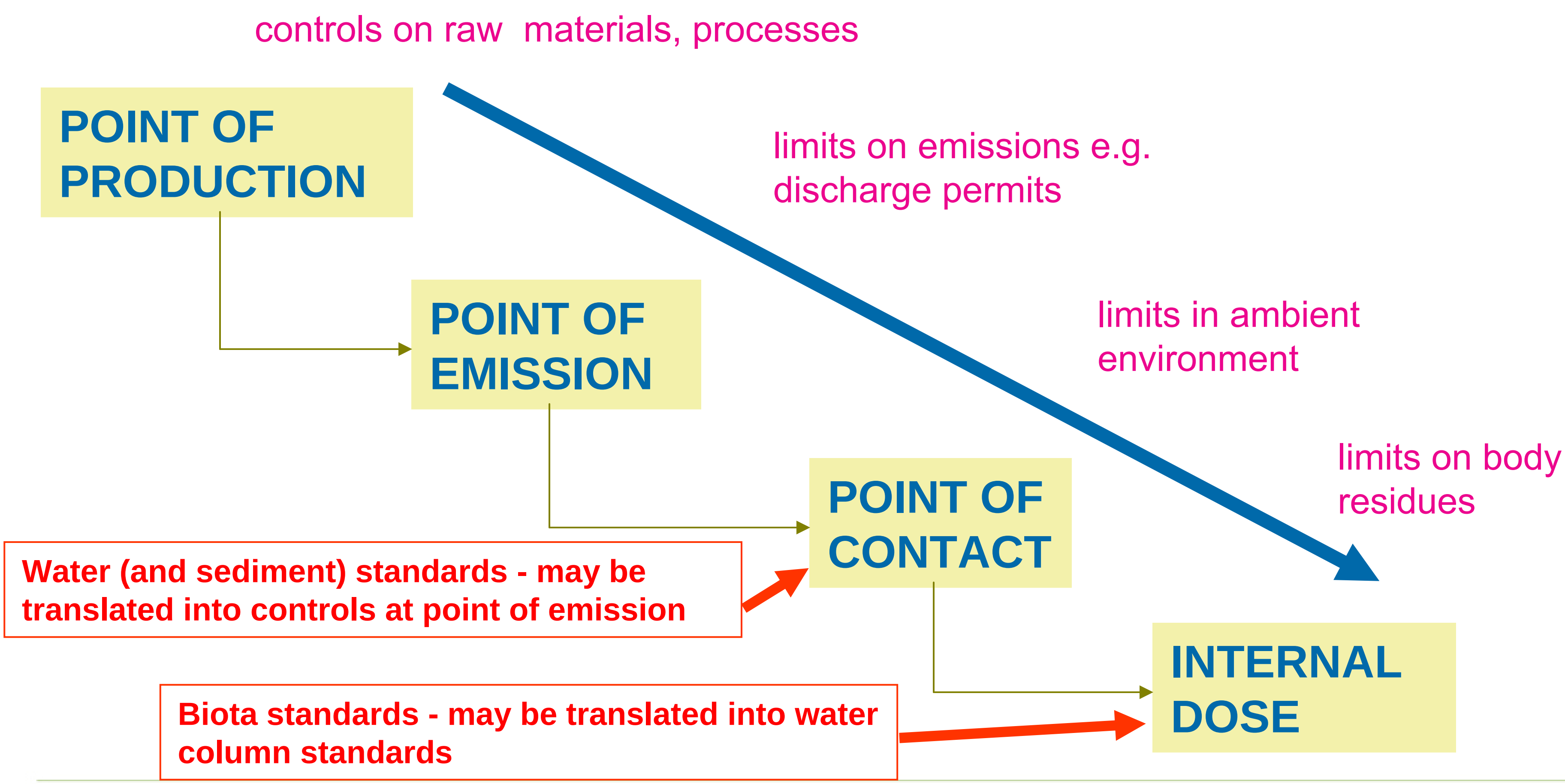
PRIORITY HAZARDOUS SUBSTANCES

Substances selected and EQS applied at EU level

SPECIFIC POLLUTANTS

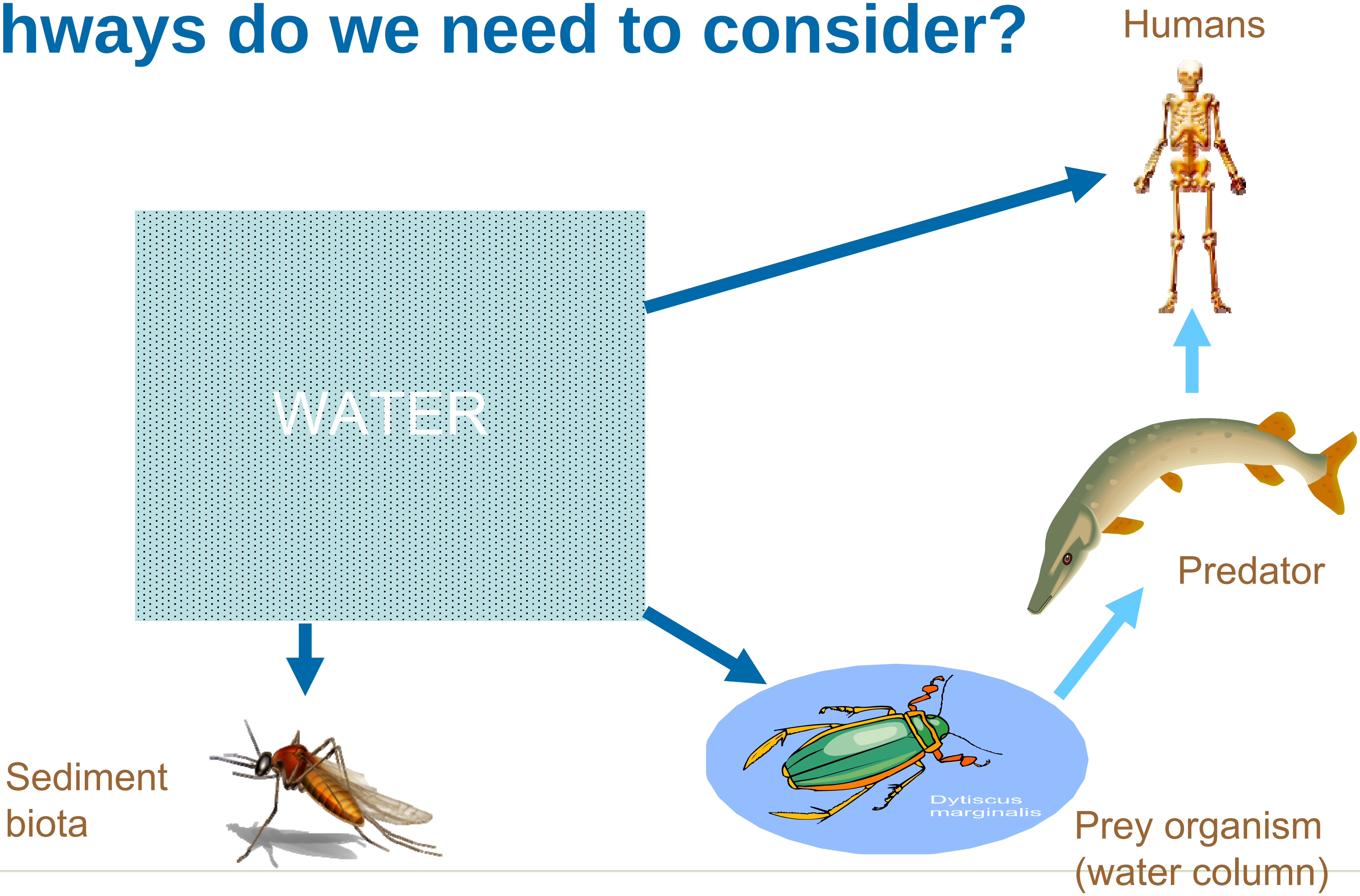
Substances identified and EQS derived by individual Member States

Points of protection



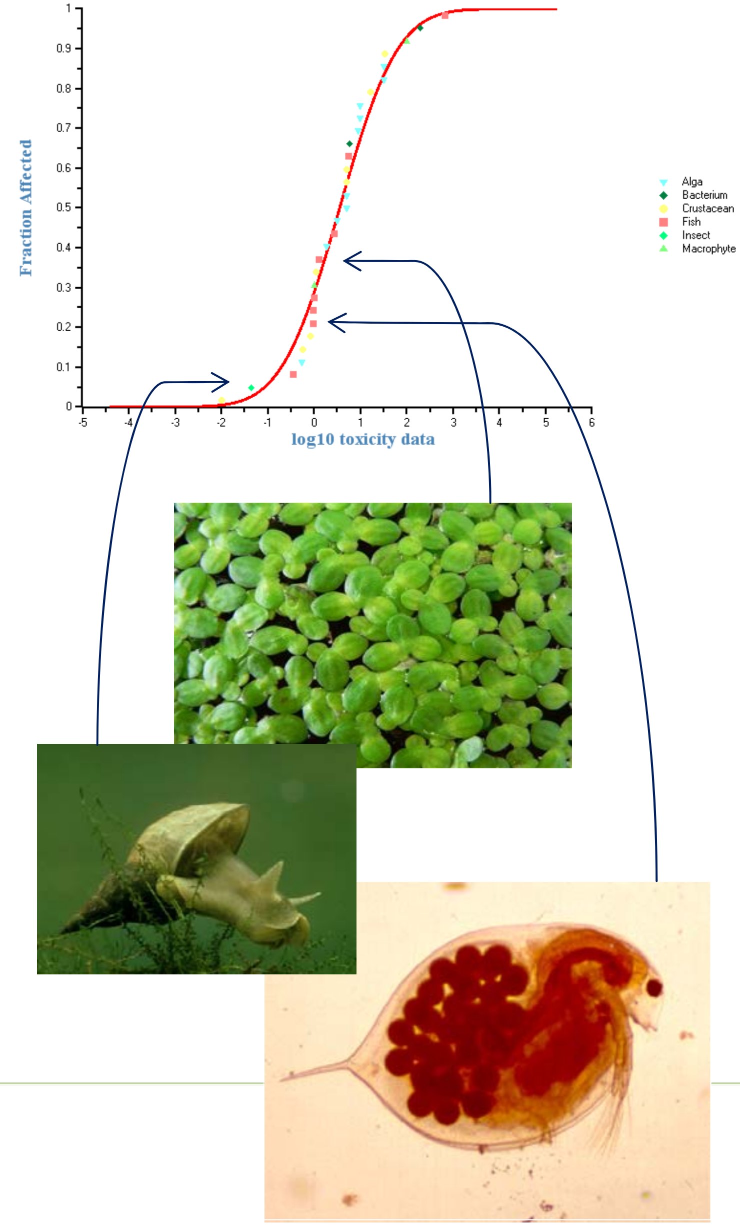
Deriving EQSs

Deriving EQSs - what receptors and pathways do we need to consider?

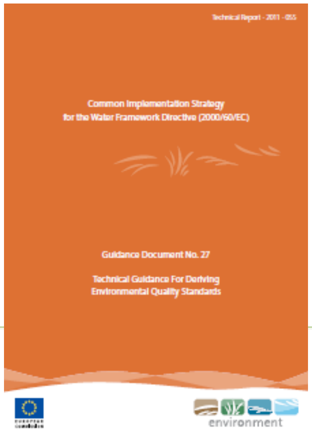


Developing EQSs

- EQSs are intended to protect aquatic life and humans from exposure via water
- Conventionally based on collection, review and analysis of laboratory toxicity data
- Assessment factors to account for uncertainty (e.g. gaps in the data)
- Field data can help reduce uncertainty



EQS Technical Guidance 2011



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EQS derivation - data sources


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A NOVEL METHOD USING CYANOBACTERIA FOR ECOTOXICITY TEST OF VETERINARY ANTIMICROBIAL AGENTS

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Abstract—The effect of antimicrobial agents for veterinary use on the growth of cyanobacteria was investigated by measuring minimum inhibitory concentration, medium effective concentration (EC50), and no-observed-effect concentration of seven antimicrobial agents for eight cyanobacteria. The results demonstrated that the seven antimicrobial agents, even at low concentrations, inhibited the growth of cyanobacteria. *Microcystis aeruginosa* and *Synechococcus* sp. had the highest sensitivity to the antimicrobial agents used in the present study. It is considered that the utilization of cyanobacteria would enable easy and highly sensitive assessment of the toxicity of such chemicals as antimicrobial agents. We suggest that cyanobacteria be used for ecotoxicity test in addition to the hitherto established method that uses green algae.

Keywords—Antimicrobial agent Cyanobacterium Ecotoxicity test Algal toxicity

INTRODUCTION

In recent stock farming programs, livestock is intensively farmed, and large quantities of antimicrobial agents are used to prevent and treat infectious diseases. Part of these antimicrobial agents administered to livestock is excreted through the feces and urine without being metabolized and/or inactivated [1,2]. Therefore, it is expected that these active antimicrobial agents are released directly to the soil environment of livestock farms [3]. On the other hand, in livestock barns in Japan, excreta must be collected and cleaned up via a wastewater treatment process such as the activated sludge process. However, active antimicrobial agents decrease the ability of microorganisms in the activated sludge to degrade organic compounds and often remain in the effluent from the treatment process [4–7]. After discharging treated water, residual antimicrobial agents are released to the aquatic environment, such as rivers and lakes. Antimicrobial agents have been detected in the effluent of sewage treatment plants [7–9] and in the environment [7,8,10–12]. The antimicrobial agents that were released to soil and aquatic environments may influence microorganisms in the area and affect the ecosystem [13]. The emergence of drug-resistant strains of bacteria could be partly due to antimicrobial agents administered to livestock. We previously investigated the effect of antimicrobial agents on such green algae as *Pseudokirchneriella subcapitata* ATCC 22662 (*Selenastrum capricornutum*) and *Chlorella vulgaris* ATCC 16487 and found that these algae were sensitive to some of the antimicrobial agents [14].

In the present study, we attempted to develop a method that uses cyanobacteria for ecotoxicity test of antimicrobial agents. We focused on the microorganisms of this division because antimicrobial agents for veterinary use, particularly antibiotics, have a significant effect on prokaryotic microorganisms such as cyanobacteria, which play the role of a primary producer in the ecosystem and inhabit various hydrospheres. Therefore, the possibility of cyanobacteria coming into contact with antimicrobial agents used in animals is high. The effect of antimicrobial agents on the growth of cyanobacteria was investigated. Then, based on the results, the importance of ecotoxicity test using cyanobacteria was discussed.

MATERIALS AND METHODS

Microorganisms and culture media

Anabaena cylindrica (NIES-19), *Anabaena variabilis* (NIES-23), *Microcystis aeruginosa* (NIES-44), and *Microcystis wesenbergii* (NIES-107) were obtained from the National Institute for Environmental Studies (NIES, Tsukuba, Ibaraki, Japan); *Nostoc* sp. (PCC 7120) and *Synechococcus* sp. (PCC 7002) were from the Pasteur Culture Collection of Cyanobacteria (PCC, Paris, France); *Synechococcus leopoldensis* (IAM M-6) was from the Institute of Applied Microbiology Culture Collection (IAM, Bunkyo-ku, Tokyo, Japan); and *Anabaena flos-aquae* (ATCC 29413) was from the American Type Culture Collection (ATCC, Manassas, VA, USA). *Anabaena cylindrica*, *A. variabilis*, and *A. flos-aquae* were cultured in modified Detmer medium (MDM) [15] in which 10 mg of $Fe_2(SO_4)_3 \cdot nH_2O$ and 27 mg of ethylenediaminetetraacetic acid (EDTA) were used instead of $FeSO_4 \cdot 7H_2O$, and the medium pH was adjusted to 7.5. *Microcystis aeruginosa*, *M. wesenbergii*, and *Nostoc* sp. were cultured in the medium described previously [16], the pH of which was adjusted to 8.5. *Synechococcus* sp. and *S. leopoldensis* were cultured in a medium consisting of NaCl (18 g/L), $MgSO_4 \cdot 7H_2O$ (5 g/L), $NaNO_3$ (1 g/L), tris(hydroxymethyl)aminomethane (1 g/L), KCl (0.6 g/L), $CaCl_2 \cdot 2H_2O$ (0.37 g/L), KH_2PO_4 (50 mg/L), Na_2EDTA (30 mg/L), $FeCl_3$ (8 mg/L), $MnCl_2 \cdot 2H_2O$ (4.3 mg/L), $ZnCl_2$ (0.32 mg/L), MoO_3 (0.03 mg/L), $CoCl_2 \cdot 6H_2O$ (0.012 mg/L), $CuSO_4 \cdot 5H_2O$ (0.003 mg/L), boric

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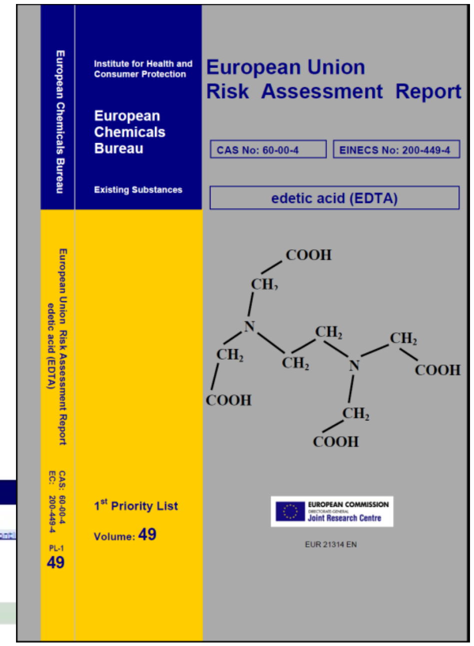
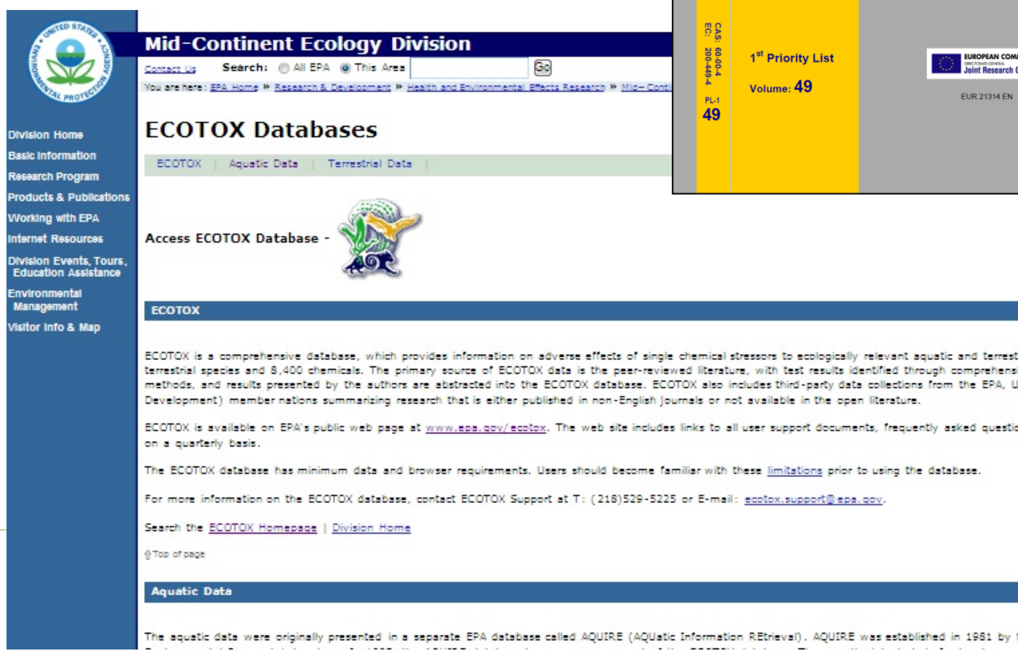
All available data for any taxonomic group should be considered, provided the data meet quality requirements for relevance and reliability

EU EQS Technical Guidance, 2011

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ECOTOX Databases

ECOTOX | Aquatic Data | Terrestrial Data |

Access ECOTOX Database -

ECOTOX

ECOTOX is a comprehensive database, which provides information on adverse effects of single chemical stressors to ecologically relevant aquatic and terrestri terrestrial species and 8,400 chemicals. The primary source of ECOTOX data is the peer-reviewed literature, with test results identified through comprehensiv methods, and results presented by the authors are abstracted into the ECOTOX database. ECOTOX also includes third-party data collections from the EPA, U. Development) member nations summarizing research that is either published in non-English journals or not available in the open literature.

ECOTOX is available on EPA's public web page at www.epa.gov/ecotox. The web site includes links to all user support documents, frequently asked questior on a quarterly basis.

The ECOTOX database has minimum data and browser requirements. Users should become familiar with these limitations prior to using the database.

For more information on the ECOTOX database, contact ECOTOX Support at T: (218)529-5225 or E-mail: ecotox.support@epa.gov.

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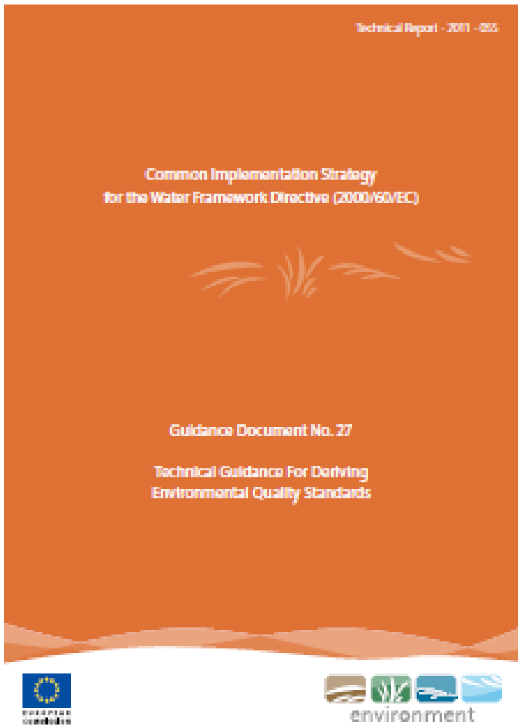
Aquatic Data

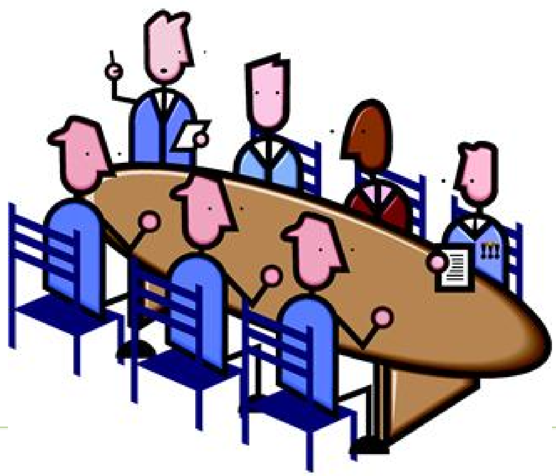
The aquatic data were originally presented in a separate EPA database called AQUIRE (AQUatic Information REtrieval). AQUIRE was established in 1981 by th

Promoting consistency



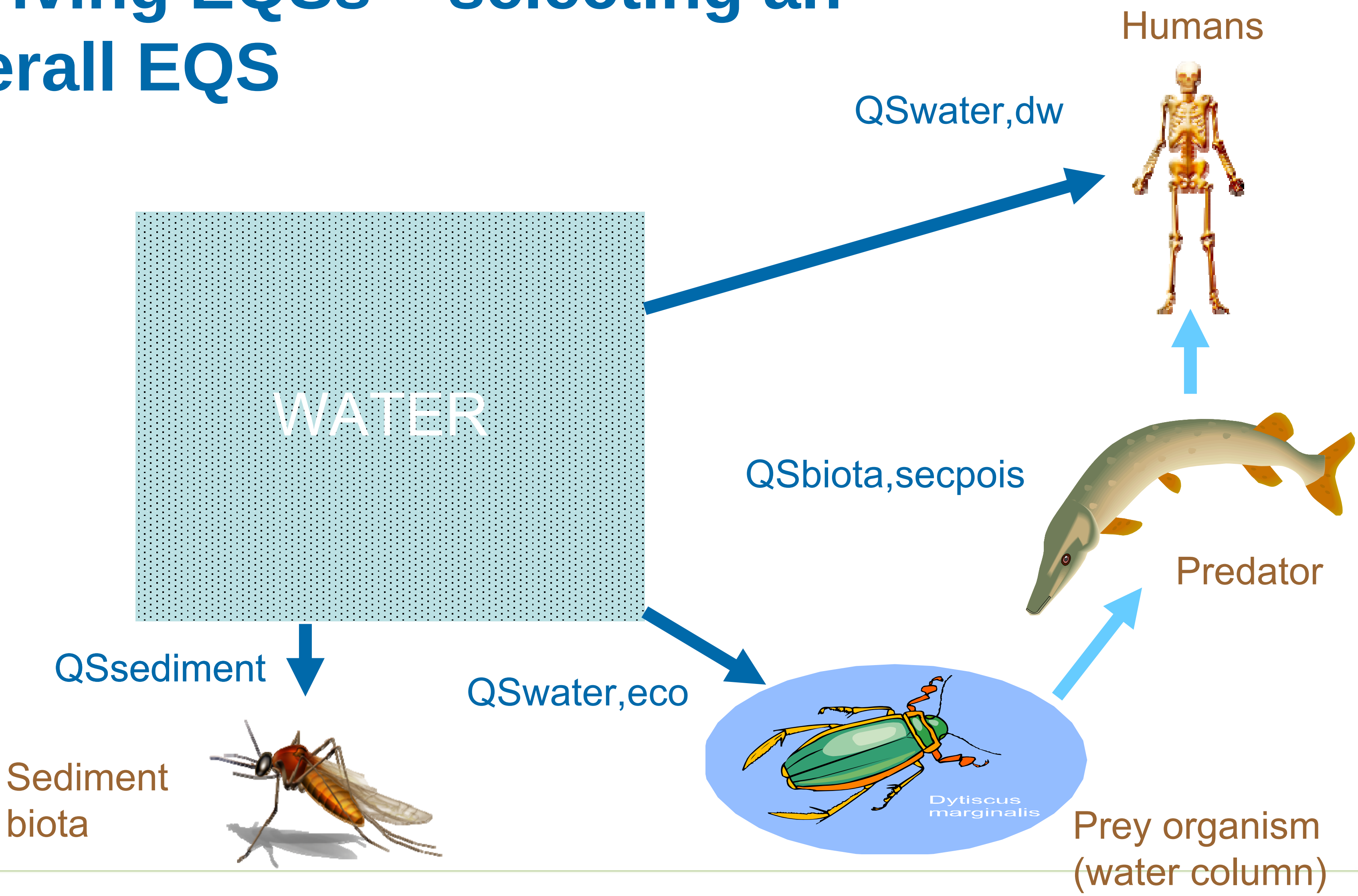
EQS Technical Guidance, 2011

Training e.g. Somma Lombardo, 2011

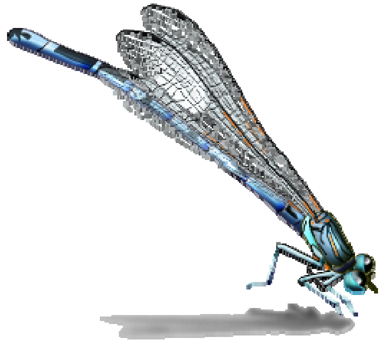


Informal discussions around ‘difficult’ substances and generic issues e.g. ‘Multilateral’ group

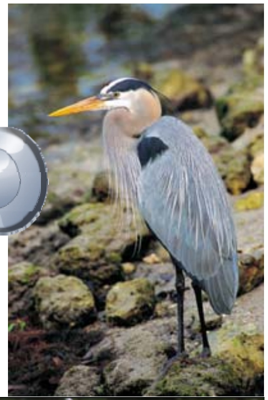
Deriving EQSs – selecting an overall EQS



Selecting an overall EQS



- Don’t need to consider all receptors and pathways for every substance
- Depends on individual substance properties
  - Physicochemical (bioaccumulation or log Kow)
  - Toxicity to mammals and birds
- ‘Triggers’ to determine whether an assessment is needed or not
- Usually several assessments, each resulting in its own QS
- Most stringent (i.e. most sensitive receptor) adopted as ‘overall’ EQS



Selecting the assessments to be performed

Water soluble, polar e.g. glyphosate, cyanide

Water (direct ecotoxicity)	**Sediment**	**Predators** (sec pois)	**Human health** (fishery products)	**Human health** (drinking water)

Are the triggers met?

QSwater,eco	no assessment	no assessment	no assessment	QSwater,dw

Selecting the assessments to be performed

Lipophilic, non-polar e.g. PAH, quinoxyfen

Water (direct ecotoxicity)	**Sediment**	**Predators** (sec pois)	**Human health** (fishery products)	**Human health** (drinking water)

Are the triggers met?

QSwater,eco	QSsediment	QSbiota,sec pois	QSbiota,hh	QSwater,dw

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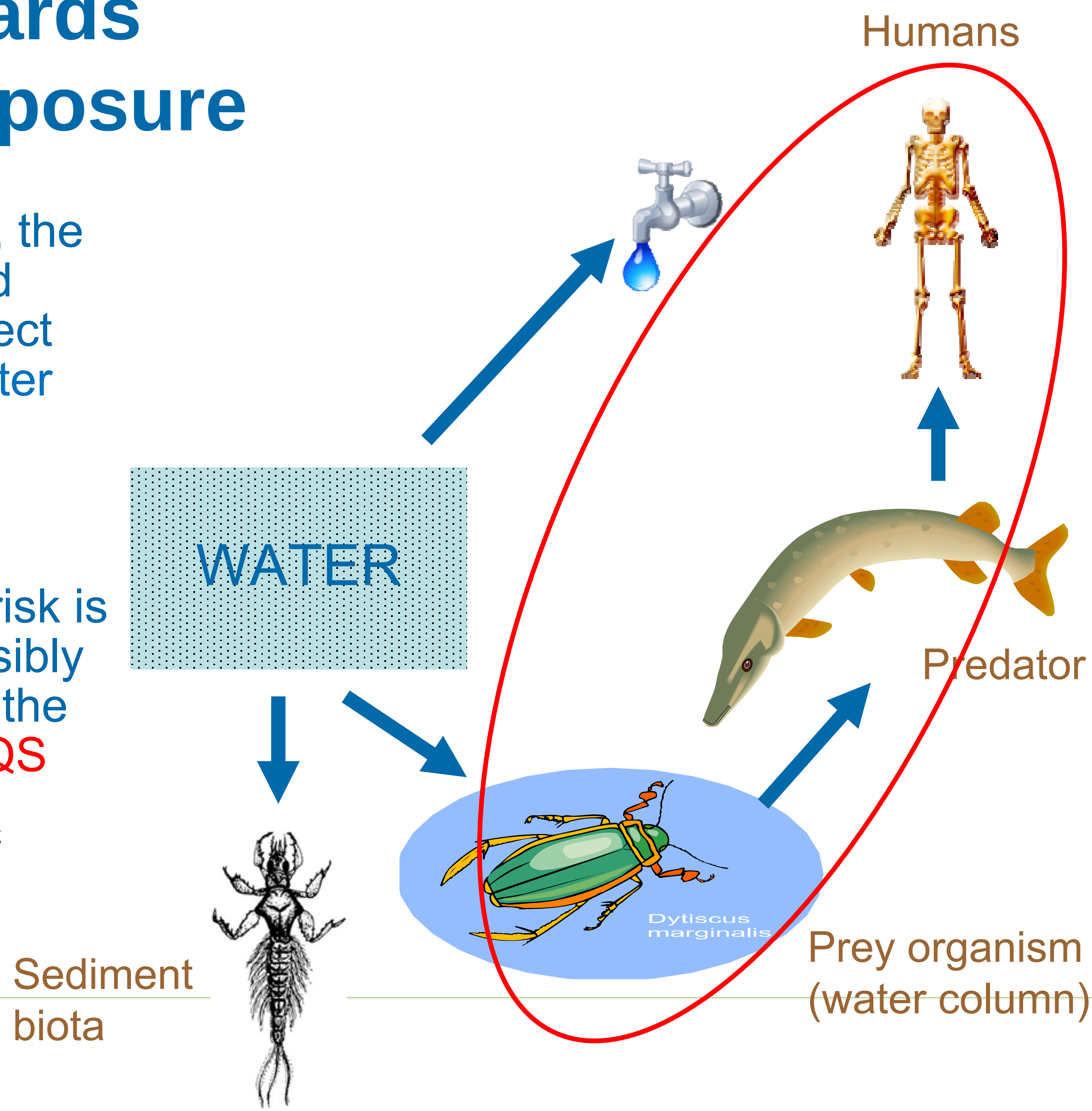
Setting standards – routes of exposure

- For many substances, the main risk to plants and animals is through direct toxicity in water → water column EQS
- But for lipophilic substances that bioaccumulate, main risk is to predators (and possibly humans) exposed via the food chain → biota EQS
- More on this from Eric Verbruggen ....



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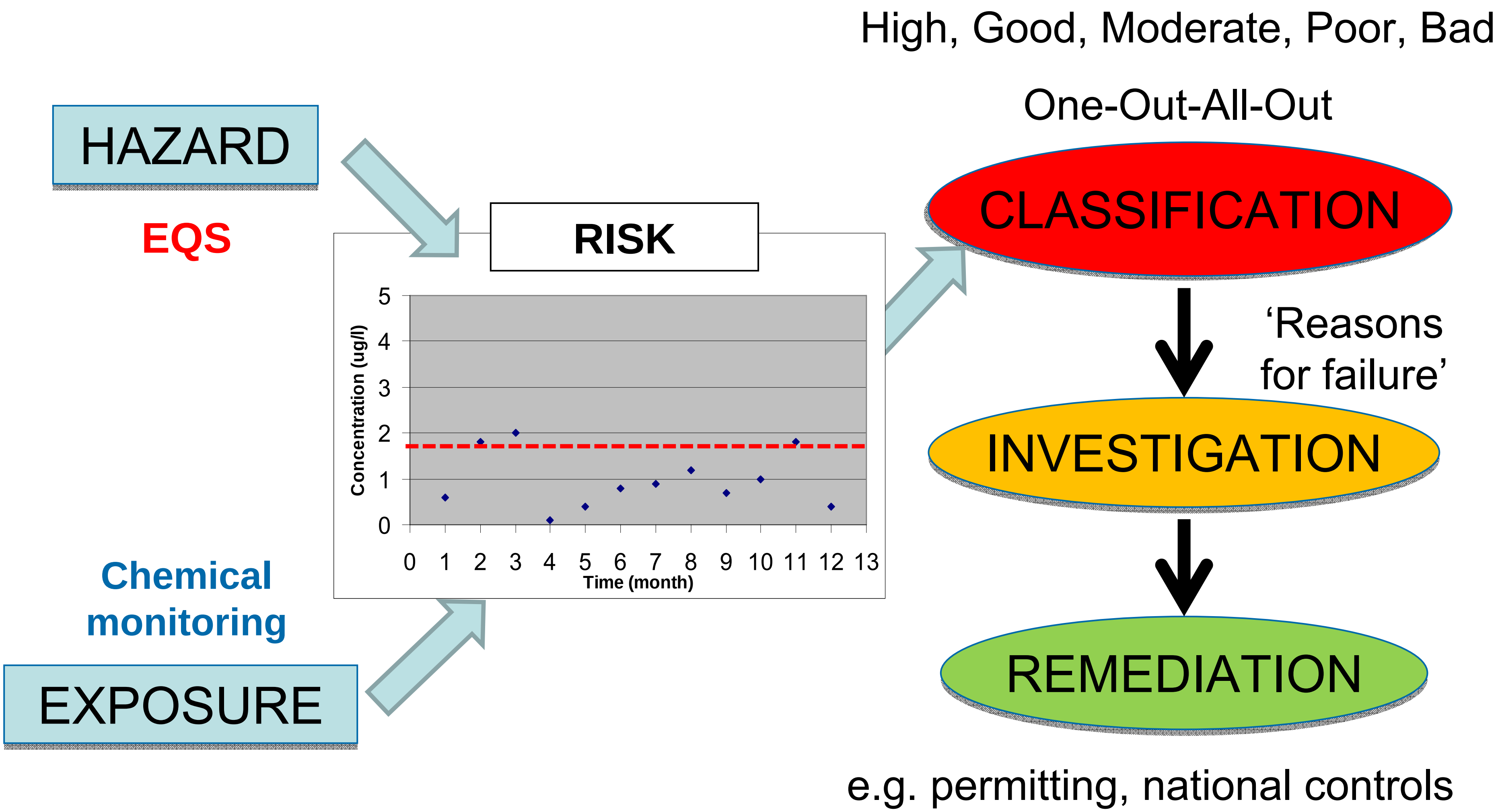
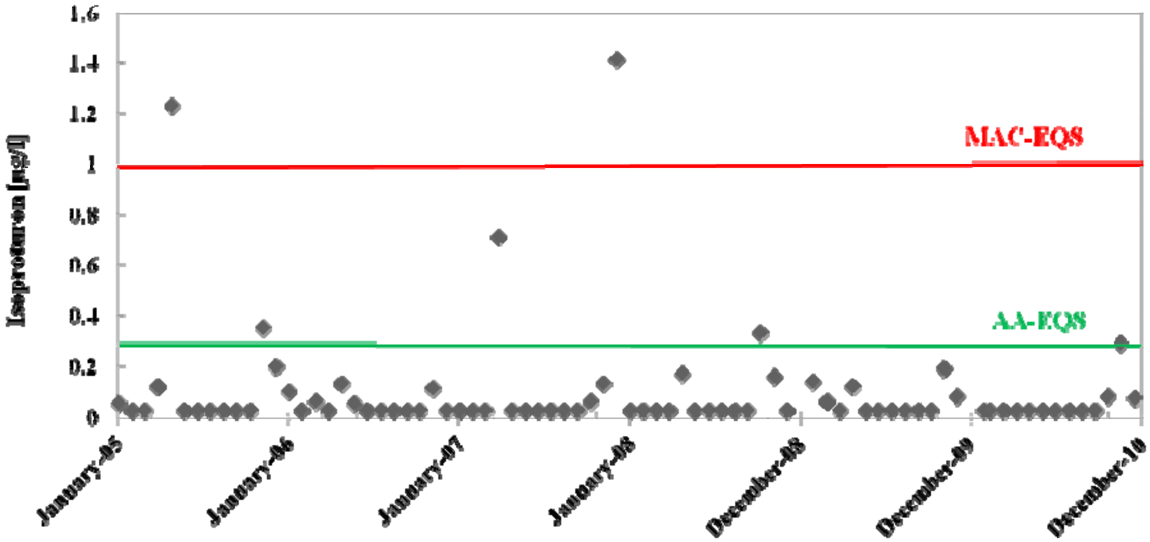
Implementing EQSs

How are EQSs used?



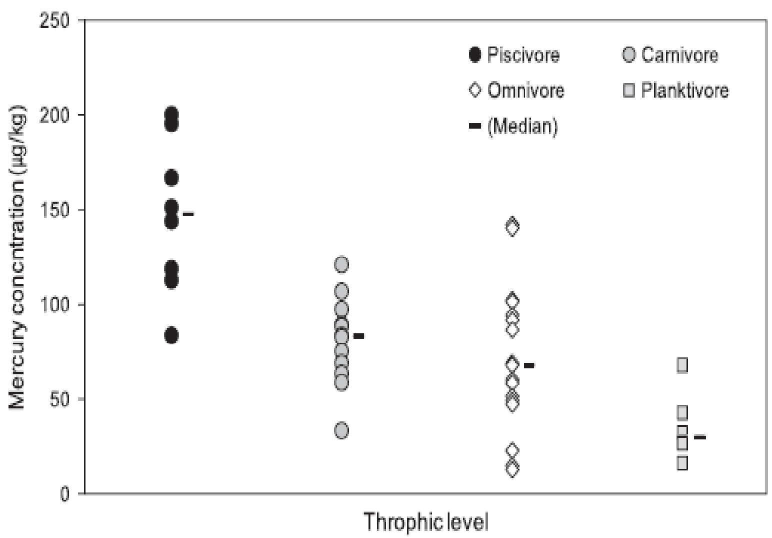
Assessing compliance – water column EQS



- Chemical considerations
  - EQSs based on dissolved concentrations (bioavailable concentrations for some metals) but sampling often based on whole water sample
  - Metals require determinations of dissolved metal, DOC, pH and [Ca] in order to estimate bioavailable concentration
- Statistical considerations
  - Absolute limits are subject to bias ... AA and 95%iles preferred
  - Discrete (spot) sampling provides estimate of variance

Assessing compliance – biota EQS

- EQS_{biota} developed for 11 substances humans and wildlife
- EQSD stipulates ‘fish’ for EQS_{biota}
- No guidance on choice of species, age class
- Can estimate equivalent water concentration using BCF



Difficult to use equivalent water concentration as compliance metric – analytical sensitivity, uncertainty about BCF

MSs likely to adopt different approaches - risk of bias

Problems with acquiring sufficient sample and animal welfare concerns

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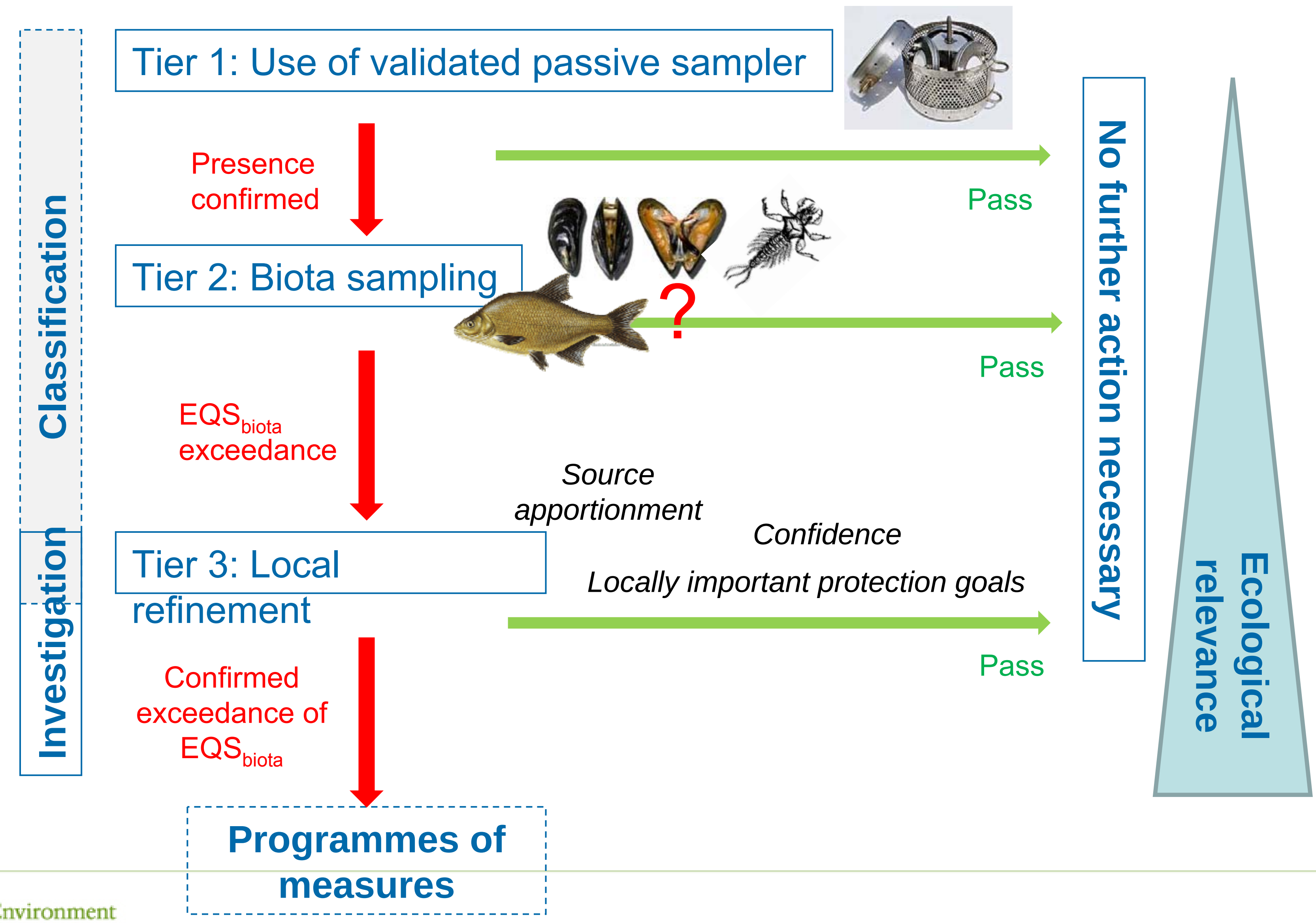
Implementing biota standards – a possible way forward?



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Possible roles for passive samplers?

Regulatory role	Required characteristics
Screening	• Detect wide range substances • Highlight locations at risk
EQS compliance assessment - Water - Biota	 • Quantify concentrations of substances with range of physico-chemical properties • Surrogate methods acceptable but must be able to infer biota concentrations • Reproducible (within and between labs)
Trend monitoring	• Absolute concentrations necessary? • Reproducible
Investigations	• Diagnostic? • Accurate and reproducible (high confidence if remedial measures are required)
Permitting	?

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Thank you for your attention

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