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Bi2003 Ecotoxicology

Ecotoxicological bioassays

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Content

- Introduction – what, why, how, concept
- Types of bioassays
- Ecotoxicological bioassays' design and results
- Aquatic bioassays - examples
- Soil bioassays – examples
- **Use of bioassays in praxis**

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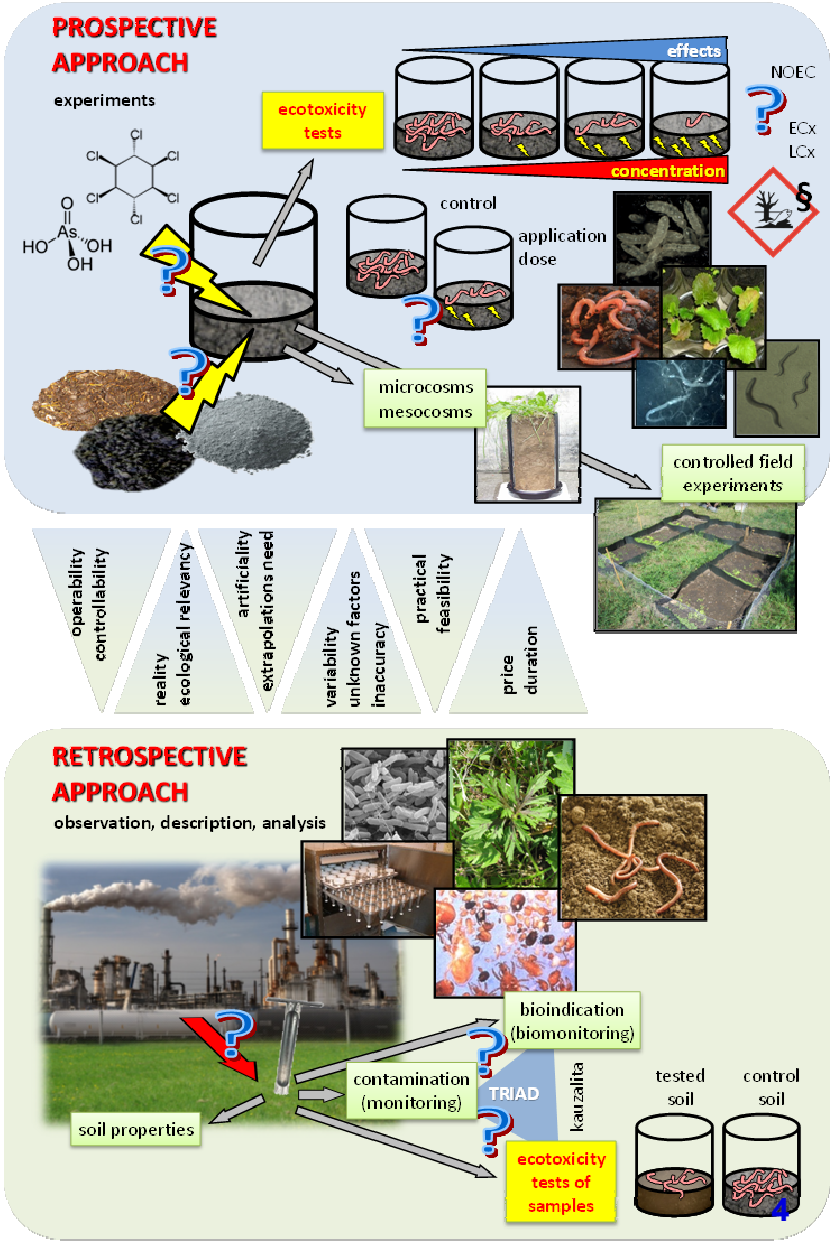
Use in praxis

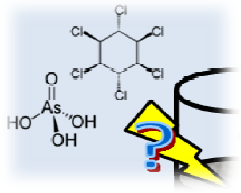
Use of bioassays in praxis

- **„praxis“** = environmental protection, regulation, legislation ...
- use of bioassays depends on: **are they officially required by legislation/regulation??**
- this is changing in time! – legislation development
- bioassays are used in **both prospective** (MORE) **and retrospective** (LESS) **approaches**
- in prospective approach, very often in **concept of RISK ASSESSMENT**



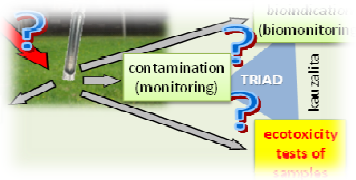
Use of bioassays in praxis

- **mainly for hazard identification / characterization of chemical substances including important specific groups of pesticides, biocides, pharmaceuticals, cosmetics ...**
  properties of same chemical (unique CAS No.), including ecotox, are constant
  including the setting of **limits for pollution**
  use of bioassays in legislation



- less (but increasing) for **complex samples (materials) in prospective approach** (e.g. before their use) – wastes, sewage sludge, fertilizers etc.
  each sample is different (e.g. sewage sludge sampled different days) – new testing needed
  legislative use is rare (only national)

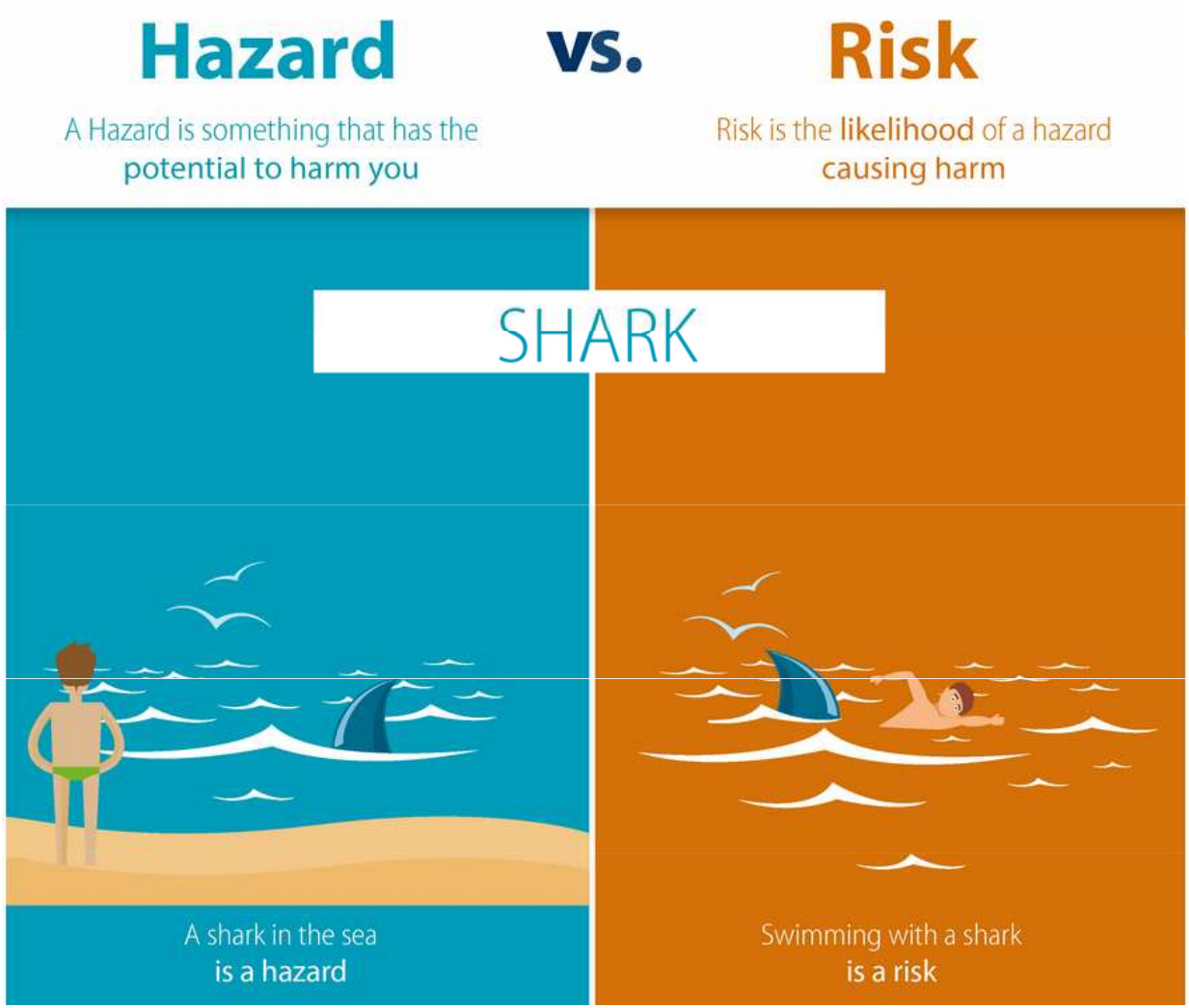
- less (but increasing) for **complex environmental samples** (water, soil, sediment ...) in retrospective approach (diagnostics)
  legislative use is rare (only national)



Risk assessment concept

- ecotoxicological bioassays are used to quantify **HAZARD (i.e. kind of property of chemicals / materials ...)**

- **RISK is then result of combination of hazard and exposure – probability that hazard will occur**



Risk assessment concept

HAZARDS

- are also physico-chemical and toxicological and other harmful properties
- ecotoxicological hazards are derived from the results of bioassays:

1. direct use of **LCx/ECx, NOEC, LOEC ...**
2. tuning these values towards **PNEC (Predicted No Effect Concentration)**, often by the most sensitive result + using so called **uncertainty factors**

Risk assessment concept

- **PNEC derivation from ecotoxicity data**
- objective is to protect real ecosystems, safe concentration

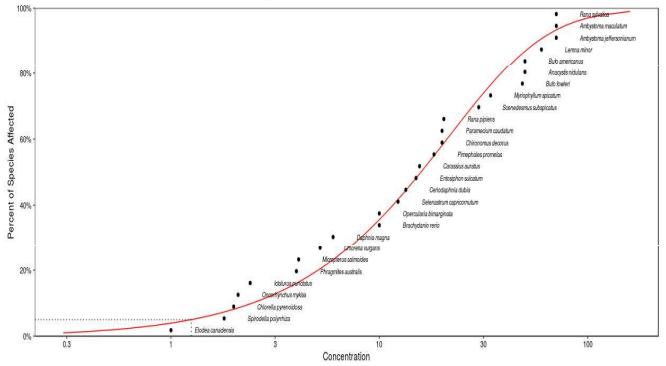
➔ need to **extrapolate** bioassay results to be valid for ecosystems

- how much information we have? → uncertainty factors, 1, 10, 100, 1000

Extrapolation

Data	Assessment factor
L(E)C50 short-term toxicity tests	1000
NOEC for 1 long-term toxicity test	100
NOEC for additional long-term toxicity tests of 2 trophic levels	50
NOEC for additional long-term toxicity tests of 3 species of 3 trophic levels	10

Species sensitivity distribution (SSD)



HC5 = 95% protection level

PNEC (limits, EQS)

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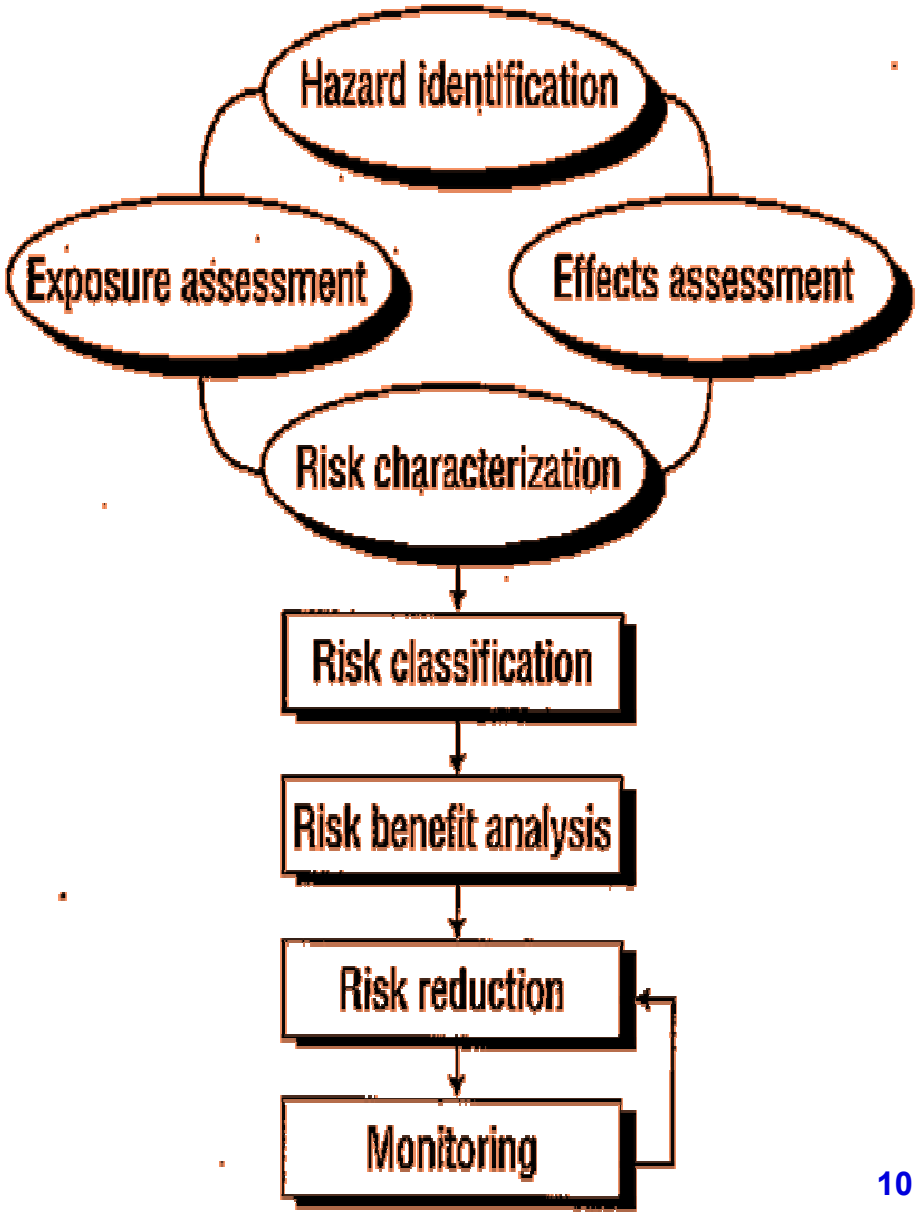
Risk assessment concept

RISK

- combines by some way
  EFFECT data (LCx/ECx, NOEC, NOAEL, PNEC etc.)
  with **EXPOSURE** (**Predicted Environmental Concentration – PEC**, Measured Environmental Concentration – MEC, etc.)
- depends on situation (factors)
- can be mitigated and managed



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Risk assessment concept

- human health risk assessment (HHRA) – human health as endpoint
- **ecological risk assessment (EcoRA, ERA)** – ecosystems, non-human organisms and populations as endpoints
- environmental risk assessment (ERA) – unclear term, involves both

Battery of bioassays

Battery of bioassays

- different organisms give different response to toxicants (sensitivity, bioavailability, exposure, metabolization ...) and also different conditions and factors in different bioassays
- **none single bioassay can give complete response !!**

- usually it is very good to combine more bioassays together = **battery**
- more and „better“ (e.g. chronic preferably to acute) bioassays used ➔ **lower uncertainty** of the finally derived hazard and risk of the tested chemical substance (lower „uncertainty factors“ in risk assessment used)

- selection should follow defined final goal of the ecotoxicity testing

Battery of bioassays

- different approaches (different purposes/legislation needs) how to do it:

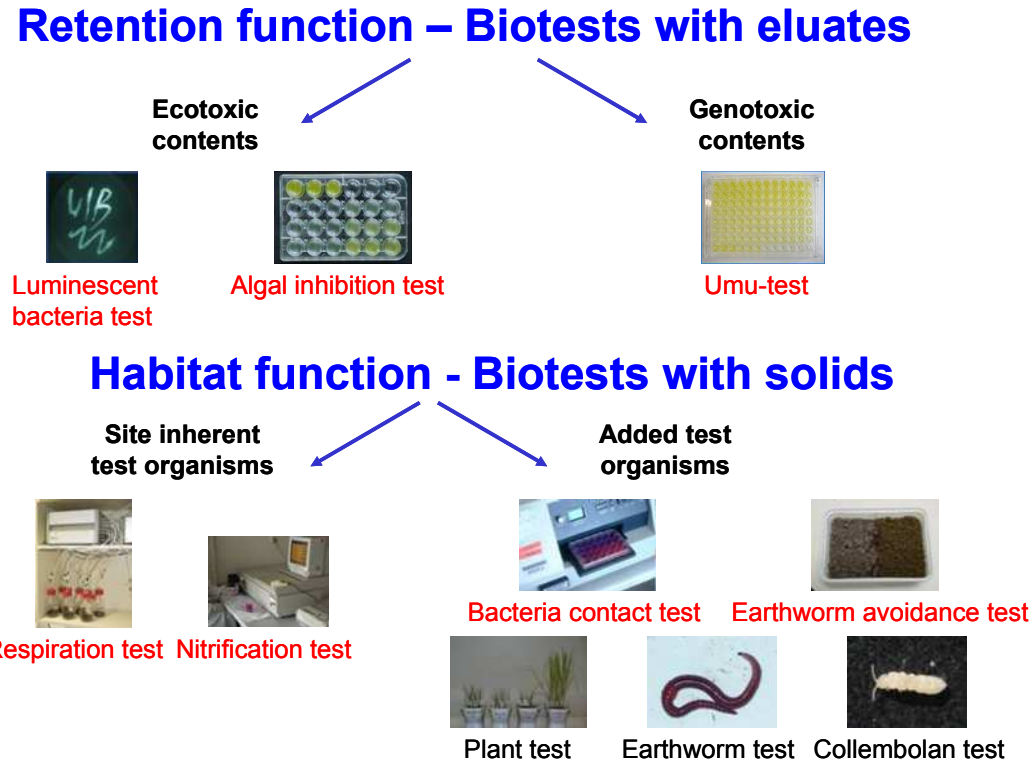
1. **combine bioassays based on some principle**
   e.g. **golden rule = combine trophic levels:** a) producer (plants), b) consumer (invertebrates, vertebrates), c) destruent (microbes)
   + also combine different routes of exposure, test duration etc.
2. mix scientific principle (1) with **practical demands** (low number of tests, quick, cheap, standardized tests ... etc.) - most common batteries in praxis:
   - algae / D. magna / fish for aquatic environment
   - earthworm (enchytraeid/springtail) / plant for soil environment
3. **tiered approach** = based on the results of previous tier its decided if next (and what next) testing will be done:
   fast screening bioassays → standardized acute bioassays → chronic/prolonged studies → field tests (so called **higher tiers**)

Battery of bioassays

Example: ISO guidelines showing how to combine bioassays for testing soils and soil-like materials

ISO 15799: Guidance on the ecotoxicological characterization of soils and soil materials

ISO 17616: Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials



Battery of bioassays

Example: Battery prepared for testing ecotoxicity of wastes

- current situation:
  - https://ec.europa.eu/environment/waste/index.htm
  - Directive 2008/98/EC on waste (Waste Framework Directive) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02008L0098-20180705
  - H14 Ecotoxicity is one of the hazardous properties of wastes
  - it is assessed based on the analysis of wastes and database data
  - no real testing with bioassays

Battery of bioassays

Example: Battery prepared for testing ecotoxicity of wastes

- there was huge ringtest focused on selection of appropriate bioassays for testing wastes (13 countries, 59 labs, 500 kg of waste tested ...)

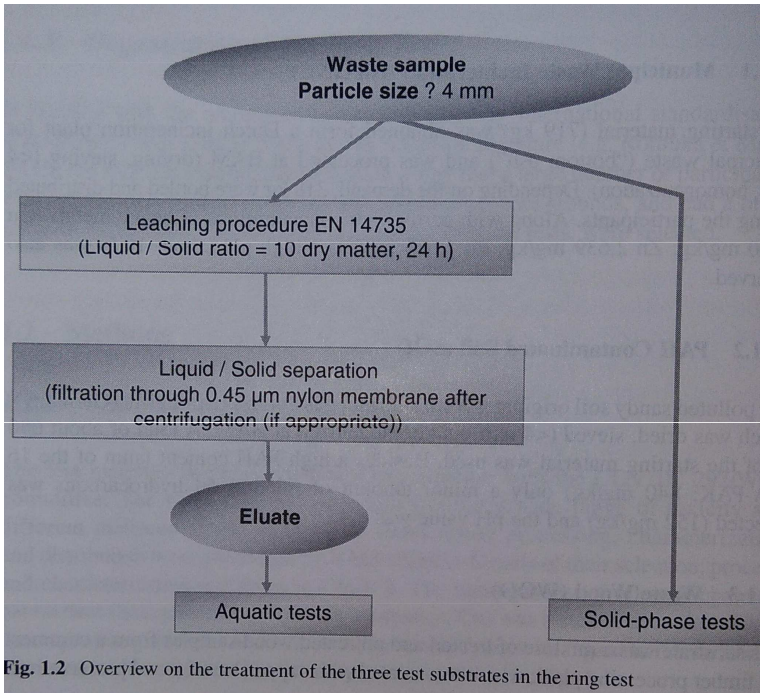


Fig. 1.2 Overview on the treatment of the three test substrates in the ring test

Table 1.1 Brief overview on the five tests belonging to the basic test battery

Name	Guideline	Species
Eluate (aquatic) waste tests		
Algae	ISO 8692 (2004)	*Desmodesmus subspicatus* or *Pseudokirchneriella subcapitata*
Daphnia	ISO 6341 (1996)	*Daphnia magna*
Luminescent bacteria	ISO 11348-1/2 (2005)	*Vibrio fischeri* (three sources, alternatively)
Solid (terrestrial) waste tests		
Earthworms (acute)	ISO 11268-1 (1997)	*Eisenia fetida* or *Eisenia andrei*
Plants	ISO 11269-2 (2004)	*Avena sativa* and *Brassica rapa*

Table 1.3 Brief overview on the ten tests belonging to the additional test battery

Name	Guideline	Species
Eluate (aquatic) waste tests		
Aquatic macrophyte	ISO 20079 (2004c)	*Lemna minor*
Rotifer	ISO/CD 20666 (2007b)	*Brachionus calyciflorus*
Sludge bacteria	ISO 10712 (1995)	*Pseudomonas putida*
Water flea	AFNOR 90-376 (2000)	*Ceriodaphnia dubia*
Umu Genotoxicity	ISO 13829 (2000)	*Salmonella choleraesius*
Solid (terrestrial) waste tests		
Earthworm reproduction	ISO 11268-2 (1998)	*Eisenia fetida* or *Eisenia andrei*
Enchytraeidae	ISO 16387 (2004d)	*Enchytraeus albidus* or *E. crypticus*
Collembola	ISO 11267 (1999)	*Folsomia candida*
Earthworm avoidance	ISO 17512-1 (2007)	*Eisenia fetida* or *Eisenia andrei*
Arthrobacter contact	DIN 38412-48 (2002)	*Arthrobacter globiformis*

Battery of bioassays

Example: Czech legislation for waste

- **H14 Ecotoxicity hazardous property determination** (decree 376/2001):
  - waste eluate 10 L/kg (EN 12457-4)
  - **if any of these tests has $LC(EC,IC)_{50} \leq 10$ ml/L, the waste sample is H14**
    - ISO 6341 Daphnia magna acute
    - ISO 8692 Algae growth
    - ISO 7346-2 Fish acute test
    - mustard Sinapsis alba germination and root growth

Battery of bioassays

Example: Czech legislation for waste

- **ecotoxicity testing for waste use on land surface or landfilling** (decree 294/2005):
  - waste eluate 10 L/kg (EN 12457-4)
  - use of waste for for closing landfills, forming protective layer, sealing layer or reclamation layer of landfill **is allowed if:**
    - Fish acute test: no mortality or behavior change
    - Daphnia magna acute test: < 30% imobilization compatred to control
    - Algae growth test: < 30% inhibition compared to control
    - Sinapis alba germination and root elongation: < 30% inhibition compared to control

Battery of bioassays

Example: Czech legislation for dredged sediments

- before application on agricultural land
- limits are given for concentration of pollutants
- ecotoxicity bioassays and criteria are given

ISO 16387

ISO 11267

ISO 15685

11269-1

Metoda	Kritérium toxicity
Test toxicity půd a půdních materiálů na roupici *Enchytraeus crypticus*	Sediment je ekotoxický pokud počet juvenilů ve směsném vzorku je významně nižší minimálně o 50% v porovnání s kontrolou.
Test toxicity půd a půdních materiálů na chvostoskoka *Folsomia candida*	Sediment je ekotoxický pokud počet juvenilů ve směsném vzorku je významně nižší minimálně o 50% v porovnání s kontrolou.
Stanovení inhibice nitrifikace v půdách a půdních materiálech	Sediment je ekotoxický pokud nitrifikační aktivita směsi je významně nižší minimálně o 25% než vypočítaná aditivní aktivita sedimentu a referenční půdy: $\mathbf{A_m + SD_m < 0{,}75 \,.\, A_{calc}}$, kde A_m – průměrná hodnota nitrifikační aktivity ve směsném vzorku SD_m – směrodatná odchylka nitrifikační aktivity směsného vzorku A_{calc} – vypočítaná aditivní nitrifikační aktivita směsi 1:3 sedimentu a referenční půdy dle vztahu: $0{,}25 \,.\, A_s + 0{,}75 \,.\, A_r$, kde A_s – průměrná hodnota nitrifikační aktivity sedimentu A_r – průměrná hodnota nitrifikační aktivity referenční půdy
Test inhibice růstu vyšších rostlin	Sediment je ekotoxický pokud je průměrná délka kořene rostlin ve směsném vzorku významně nižší minimálně o 30% v porovnání s kontrolou.

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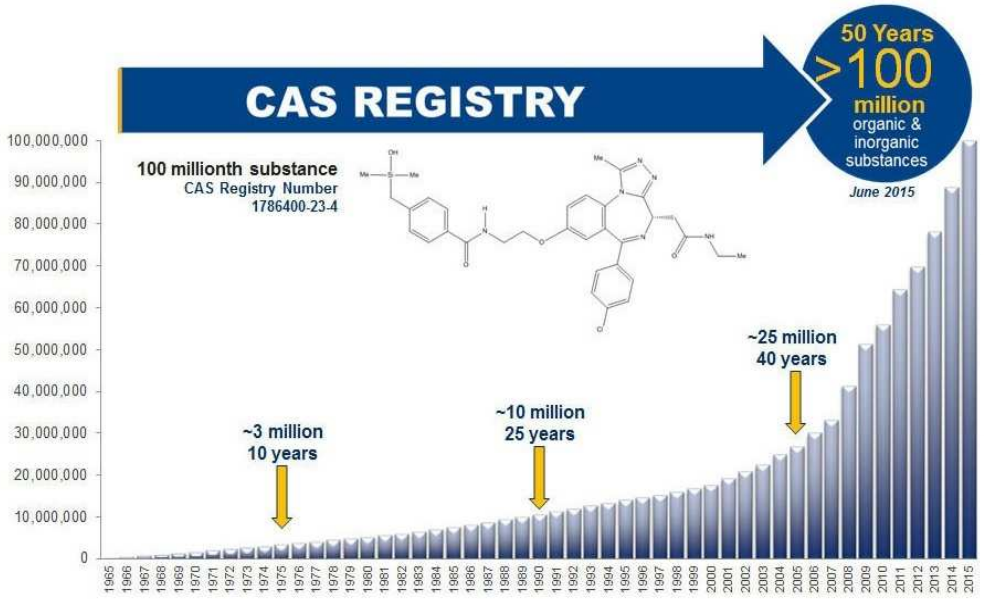
Examples of ecotoxicity bioassays use in legislation

Testing of chemical substances

- mainly „chemicals“ – they have CAS No.
- > 100 millions of chemicals known
- > 100 000 we produce and use
- HPVC = high production volume chemicals > 1000 t/y production
- „priority pollutants“ – e.g. Water Framework Directive, Stockholm Convention ...



Testing of chemical substances

- **developed legislation (EU, US ...) requires determination of ecotoxicity** (+ lot of other properties – phys-chem, toxicity ...) **before marketing and use of chemicals**
- what does it mean:
  - precise testing using standardized (OECD ...) bioassays in accredited labs with all validity requirements and quality assurance / quality control (QA/QC) measures
  - results of ecotoxicity clearly expressed avoiding any confusion or missinterpretation:
    - what parameter – ECx/LCx, NOEC ...
    - which units – mg/L, mol/L, mg/kg, % ...
    - specify clearly possible variants in procedure: i.e. D. magna, 24h, LC50 = XY mg/L
    - ...
  - results used for risk assessment of chemicals, labelling, decision making, authorizations, restrictions, management etc.

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Example 1
Labelling chemicals - GHS

GHS

Globally Harmonised System of Classification and Labelling of Chemicals (https://unece.org/about-ghs)

- „hazardous to aquatic environment“

SHORT-TERM (ACUTE) AQUATIC HAZARD

	Category 1	Category 2	Category 3
Symbol	Environment	*No symbol*	*No symbol*
Signal word	Warning	*No signal word*	*No signal word*
Hazard statement	Very toxic to aquatic life	Toxic to aquatic life	Harmful to aquatic life

LONG-TERM (CHRONIC) AQUATIC HAZARD

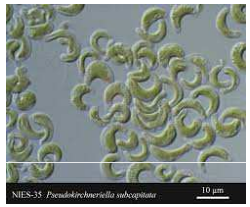
	Category 1	Category 2	Category 3	Category 4
Symbol	Environment	Environment	*No symbol*	*No symbol*
Signal word	Warning	*No signal word*	*No signal word*	*No signal word*
Hazard statement	Very toxic to aquatic life with long lasting effects	Toxic to aquatic life with long lasting effects	Harmful to aquatic life with long lasting effects	May cause long lasting harmful effects to aquatic life

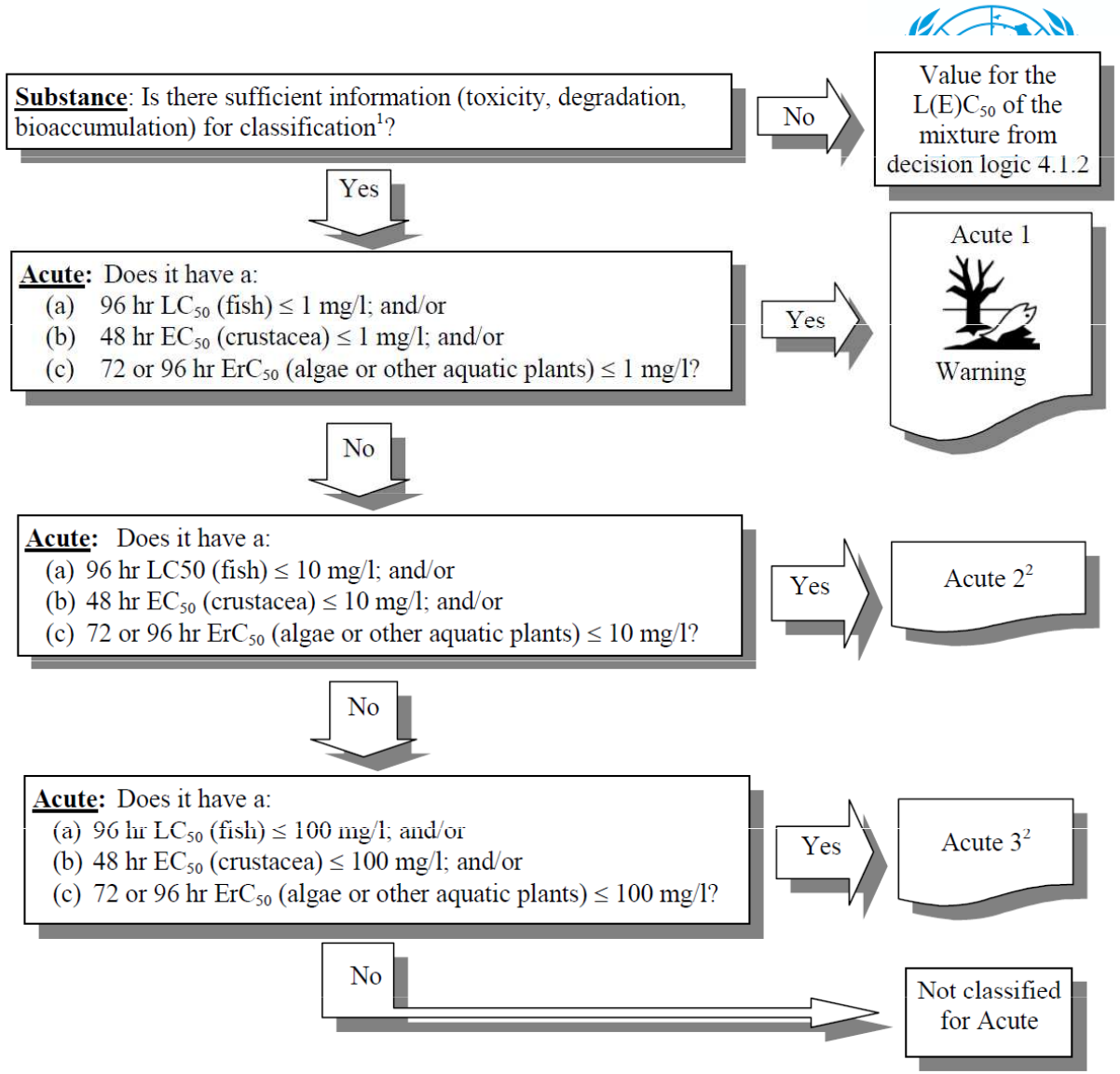
- OECD tests preferred and GLP (good laboratory praxis) should be followed
  - acute aquatic toxicity
  - chronic aquatic toxicity
  - potential for or actual bioaccumulation
  - degradation (biotic or abiotic) for organic chemicals

GHS

Acute classification categories 1 to 3

- defined on the basis of the acute toxicity data only (EC50 or LC50)
  - fish 96h (OECD 203)
  - Daphnia 48h (OECD 202)
  - algae growth inhibition test (OECD 201)





United Nations (2019): Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Eighth revised edition. https://unece.org/ghs-rev8-2019

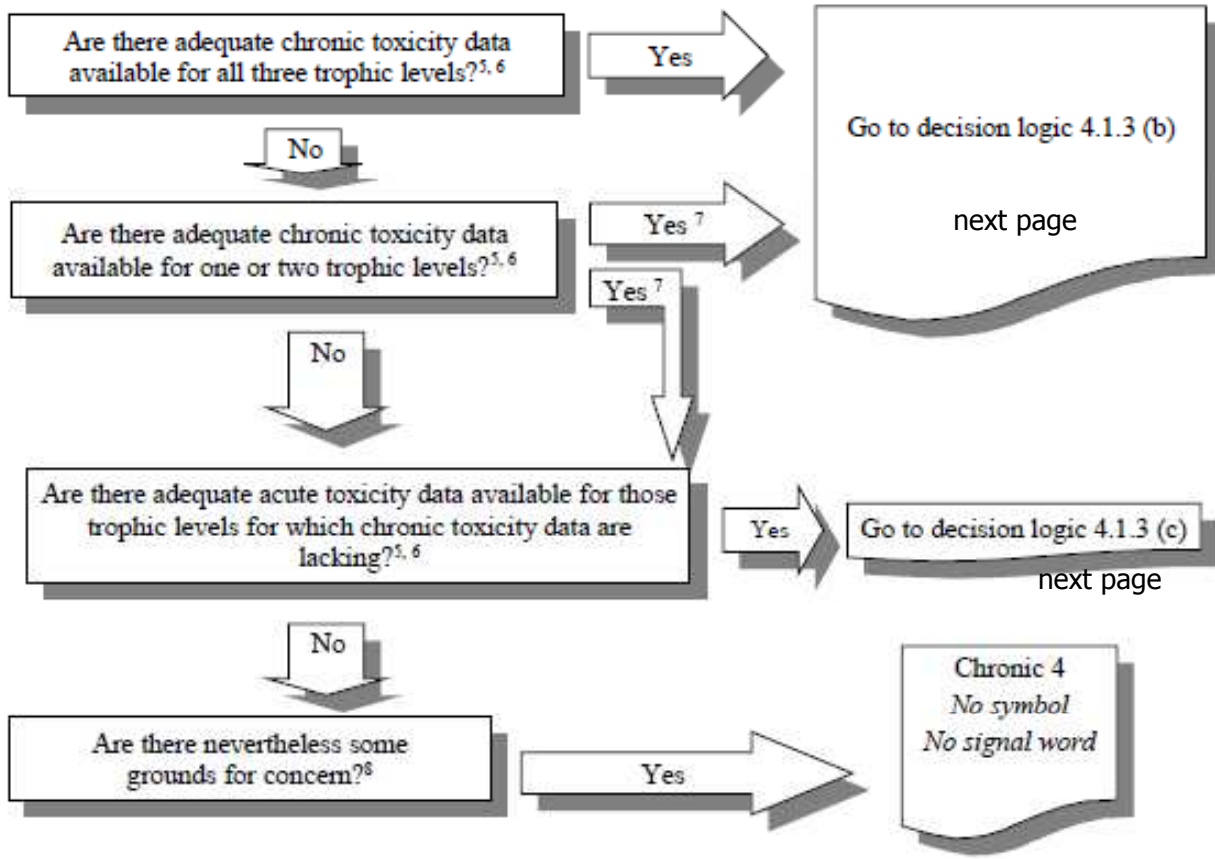
GHS

Chronic classification categories 1 to 3

- based on tiered approach ➔

1. available information on chronic toxicity
   - fish early life stage (OECD 210)
   - daphnia reproduction (OECD 211)
   - algae growth inhibition test (OECD 201)
   - NOEC or ECx (e.g. EC_{10})

2. or acute toxicity data combined with environmental fate data



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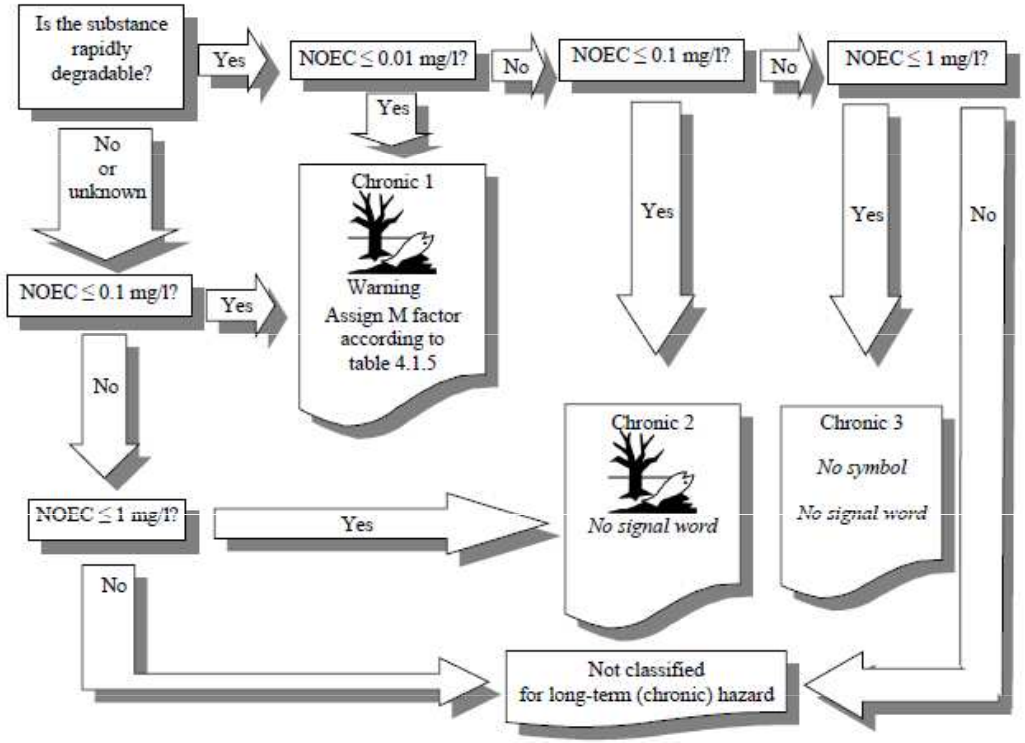
GHS

chronic classification

data available



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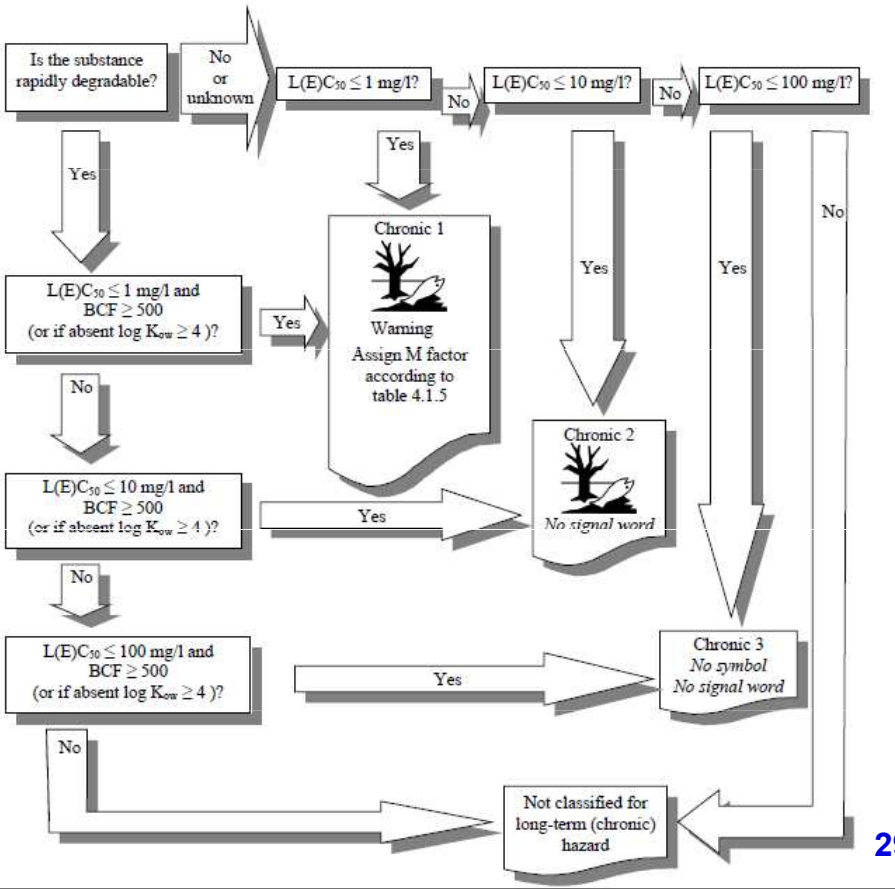

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GHS

aquatic hazards summary

Classification categories			
Short-term (acute) hazard (*Note 1*)	**Long-term (chronic) hazard** (*Note 2*)		
	Adequate chronic toxicity data available		**Adequate chronic toxicity data not available** (*Note 1*)
	Non-rapidly degradable substances (*Note 3*)	**Rapidly degradable substances** (*Note 3*)	
Category: Acute 1 $L(E)C_{50} \leq 1.00$	**Category: Chronic 1** NOEC or $EC_x \leq 0.1$	**Category: Chronic 1** NOEC or $EC_x \leq 0.01$	**Category: Chronic 1** $L(E)C_{50} \leq 1.00$ and lack of rapid degradability and/or BCF ≥ 500 or, if absent log $K_{ow} \geq 4$
Category: Acute 2 $1.00 < L(E)C_{50} \leq 10.0$	**Category: Chronic 2** $0.1 <$ NOEC or $EC_x \leq 1$	**Category: Chronic 2** $0.01 <$ NOEC or $EC_x \leq 0.1$	**Category: Chronic 2** $1.00 < L(E)C_{50} \leq 10.0$ and lack of rapid degradability and/or BCF ≥ 500 or, if absent log $K_{ow} \geq 4$
Category: Acute 3 $10.0 < L(E)C_{50} \leq 100$		**Category: Chronic 3** $0.1 <$ NOEC or $EC_x \leq 1$	**Category: Chronic 3** $10.0 < L(E)C_{50} \leq 100$ and lack of rapid degradability and/or BCF ≥ 500 or, if absent log $K_{ow} \geq 4$
	Category: Chronic 4 (*Note 4*) Example: (*Note 5*) No acute toxicity and lack of rapid degradability and BCF ≥ 500 or, if absent log Kow ≥ 4, unless NOECs > 1 mg/l		

NOTE 1: *Acute toxicity band based on $L(E)C_{50}$ values in mg/l for fish, crustacea and/or algae or other aquatic plants (or QSAR estimation if no experimental data).*

NOTE 2: *Substances are classified in the various chronic categories unless there are adequate chronic toxicity data available for all three trophic levels above the water solubility or above 1 mg/l. ("Adequate" means that the data sufficiently cover the endpoint of concern. Generally this would mean measured test data, but in order to avoid unnecessary testing it can, on a case-by-case basis, also be estimated data, e.g. (Q)SAR, or for obvious cases expert judgment).*

NOTE 3: *Chronic toxicity band based on NOEC or equivalent EC_x values in mg/l for fish or crustacea or other recognized measures for chronic toxicity.*

NOTE 4: *The system also introduces a "safety net" classification (referred to as category Chronic 4) for use when the data available do not allow classification under the formal criteria but there are nevertheless some grounds for concern.*

NOTE 5: *For poorly soluble substances for which no acute toxicity has been demonstrated at the solubility limit, and are both not rapidly degraded and have a potential to bioaccumulate, this category should apply unless it can be demonstrated that the substance does not require classification for aquatic long-term (chronic) hazards.*

United Nations (2019): Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Eighth revised edition. https://unece.org/ghs-rev8-2019

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Example 2
Chemicals – in EU

REACH

- https://ec.europa.eu/environment/chemicals/index_en.htm

- **Regulation 1907/2006, concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)**
  https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02006R1907-20200824

- responsible is **European Chemicals Agency (ECHA) https://echa.europa.eu/home**

ECHA
EUROPEAN CHEMICALS AGENCY

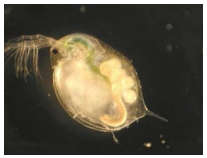
REACH

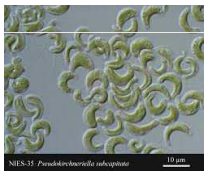
- **REACH allows to produce and use in EU only such chemical substances that are registered, evaluated and authorized in EU** (does not apply to substances with production or import below 1 t/y)
- **the risk they may pose to human health and the environment must be characterized and they must be classified accordingly**
- registration is based on **technical dossier**, which includes data on the properties of the registered substance determined by defined testing procedures
  Regulation No 440/2008, laying down test methods pursuant to Regulation No 1907/2006 (REACH)
  https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02008R0440-20191016
- for substances with production or import > 10 t/y also **Chemical Safety Report (CSR)** must be prepared involving **evaluation of hazards and risks related to production and use**

- **concept:** with increasing volume of production (1, 10, 100 and 1000 t/y) number of needed data (methods, tests, including ecotoxicity bioassays) is increasing

REACH

all substances > 1 t/y





9. ECOTOXICOLOGICAL INFORMATION

COLUMN 1 STANDARD INFORMATION REQUIRED	COLUMN 2 SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1
9.1. Aquatic toxicity	
9.1.1. Short-term toxicity testing on invertebrates (preferred species *Daphnia*) The registrant may consider long-term toxicity testing instead of short-term.	9.1.1. The study does not need to be conducted if: — there are mitigating factors indicating that aquatic toxicity is unlikely to occur for instance if the substance is highly insoluble in water or the substance is unlikely to cross biological membranes, — a long-term aquatic toxicity study on invertebrates is available, or — adequate information for environmental classification and labelling is available. For nanoforms, the study may not be waived on the basis of high insolubility in water alone. The long-term aquatic toxicity study on *Daphnia* (Annex IX, section 9.1.5.) shall be considered if the substance is poorly water soluble, or for nanoforms if they have low dissolution rate in the relevant test media.
9.1.2. Growth inhibition study aquatic plants (algae preferred)	9.1.2. The study does not need to be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur for instance if the substance is highly insoluble in water or the substance is unlikely to cross biological membranes. For nanoforms, the study may not be waived on the basis of high insolubility in water alone.

REACH

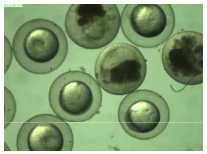
all substances
> 10 t/y

9. ECOTOXICOLOGICAL INFORMATION

COLUMN 1 STANDARD INFORMATION REQUIRED	COLUMN 2 SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1
9.1.3. Short-term toxicity testing on fish: the registrant may consider long-term toxicity testing instead of short-term.	9.1.3. The study does not need to be conducted if: — there are mitigating factors indicating that aquatic toxicity is unlikely to occur, for instance the substance is highly insoluble in water or the substance is unlikely to cross biological membranes, or — a long-term aquatic toxicity study on fish is available. For nanoforms, the study may not be waived on the basis of high insolubility in water alone. Long-term aquatic toxicity testing as described in Annex IX shall be considered if the chemical safety assessment according to Annex I indicates the need to investigate further effects on aquatic organisms. The choice of the appropriate test(s) will depend on the results of the chemical safety assessment. The long-term aquatic toxicity study on fish (Annex IX, Section 9.1.6) shall be considered if the substance is poorly water soluble, or for nanoforms if they have low dissolution rate in the relevant test media.
9.1.4. Activated sludge respiration inhibition testing	9.1.4. The study does not need to be conducted if: — there is no emission to a sewage treatment plant, or — there are mitigating factors indicating that microbial toxicity is unlikely to occur, for instance the substance is highly insoluble in water, or — the substance is found to be readily biodegradable and the applied test concentrations are in the range of concentrations that can be expected in the influent of a sewage treatment plant. For nanoforms, the study may not be waived on the basis of high insolubility in water alone.

REACH

all substances
> 100 t/y



9. ECOTOXICOLOGICAL INFORMATION

COLUMN 1 STANDARD INFORMATION REQUIRED	COLUMN 2 SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1
9.1. Aquatic toxicity	9.1. Long-term toxicity testing shall be proposed by the registrant if the chemical safety assessment according to Annex I indicates the need to investigate further the effects on aquatic organisms. The choice of the appropriate test(s) depends on the results of the chemical safety assessment.
9.1.5. Long-term toxicity testing on invertebrates (preferred species *Daphnia*), (unless already provided as part of Annex VII requirements)	
9.1.6. Long-term toxicity testing on fish, (unless already provided as part of Annex VIII requirements) The information shall be provided for one of the Sections 9.1.6.1, 9.1.6.2 or 9.1.6.3.	
9.1.6.1. Fish early-life stage (FELS) toxicity test	
9.1.6.2. Fish short-term toxicity test on embryo and sac-fry stages	
9.1.6.3. Fish, juvenile growth test	

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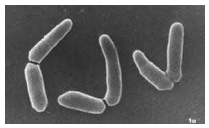
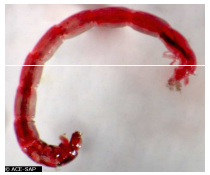
all substances
> 100 t/y



COLUMN 1 STANDARD INFORMATION REQUIRED		COLUMN 2 SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1
9.4.	Effects on terrestrial organisms	9.4. These studies do not need to be conducted if direct and indirect exposure of the soil compartment is unlikely. In the absence of toxicity data for soil organisms, the equilibrium partitioning method may be applied to assess the hazard to soil organisms. Where the equilibrium partitioning method is applied to nanoforms, this shall be scientifically justified. The choice of the appropriate tests depends on the outcome of the chemical safety assessment. In particular for substances that have a high potential to adsorb to soil or that are very persistent, the registrant shall consider long-term toxicity testing instead of short-term.
9.4.1.	Short-term toxicity to invertebrates	
9.4.2.	Effects on soil micro-organisms	
9.4.3.	Short-term toxicity to plants	

REACH

all substances
> 1000 t/y



COLUMN 1 STANDARD INFORMATION REQUIRED	COLUMN 2 SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1
9.4. Effects on terrestrial organisms	9.4. Long-term toxicity testing shall be proposed by the registrant if the results of the chemical safety assessment according to Annex I indicates the need to investigate further the effects of the substance and/or degradation products on terrestrial organisms. The choice of the appropriate test(s) depends on the outcome of the chemical safety assessment. These studies do not need to be conducted if direct and indirect exposure of the soil compartment is unlikely.
9.4.4. Long-term toxicity testing on invertebrates, unless already provided as part of Annex IX requirements.	
9.4.6. Long-term toxicity testing on plants, unless already provided as part of Annex IX requirements.	
9.5.1. Long-term toxicity to sediment organisms	9.5.1. Long-term toxicity testing shall be proposed by the registrant if the results of the chemical safety assessment indicates the need to investigate further the effects of the substance and/or relevant degradation products on sediment organisms. The choice of the appropriate test(s) depends on the results of the chemical safety assessment.
9.6.1. Long-term or reproductive toxicity to birds	9.6.1. Any need for testing should be carefully considered taking into account the large mammalian dataset that is usually available at this tonnage level.

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Regulation No 440/2008, laying down test methods pursuant to Regulation No 1907/2006 (REACH)
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02008R0440-20191016

lists the methods including procedures

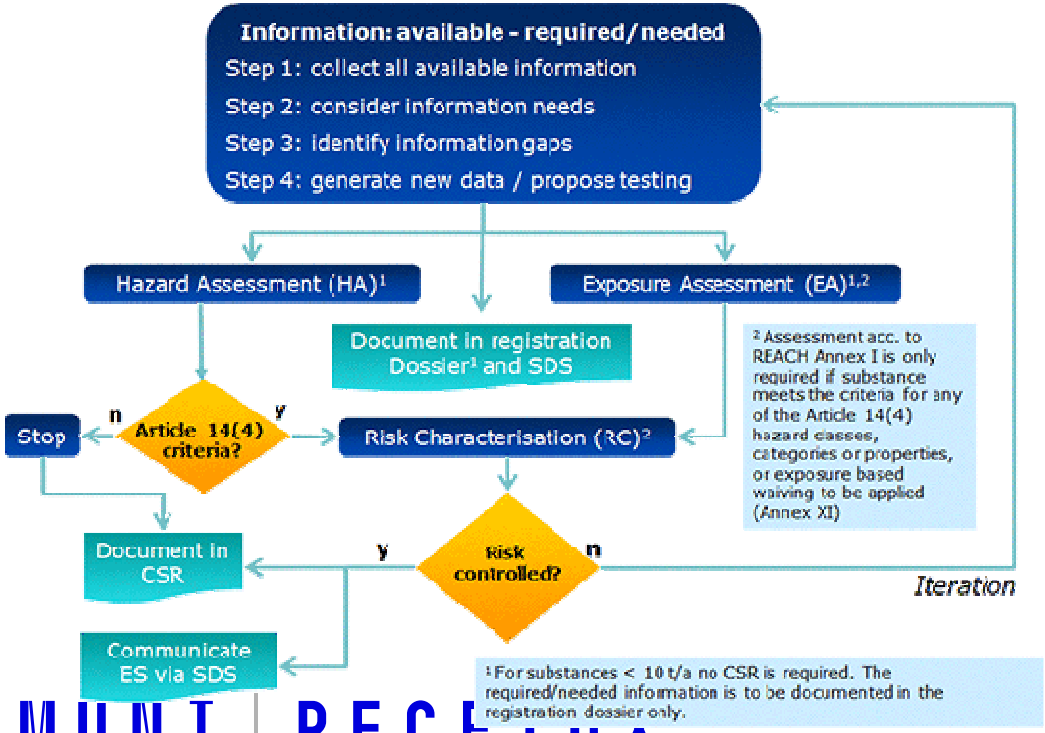
often follows OECD guidelines

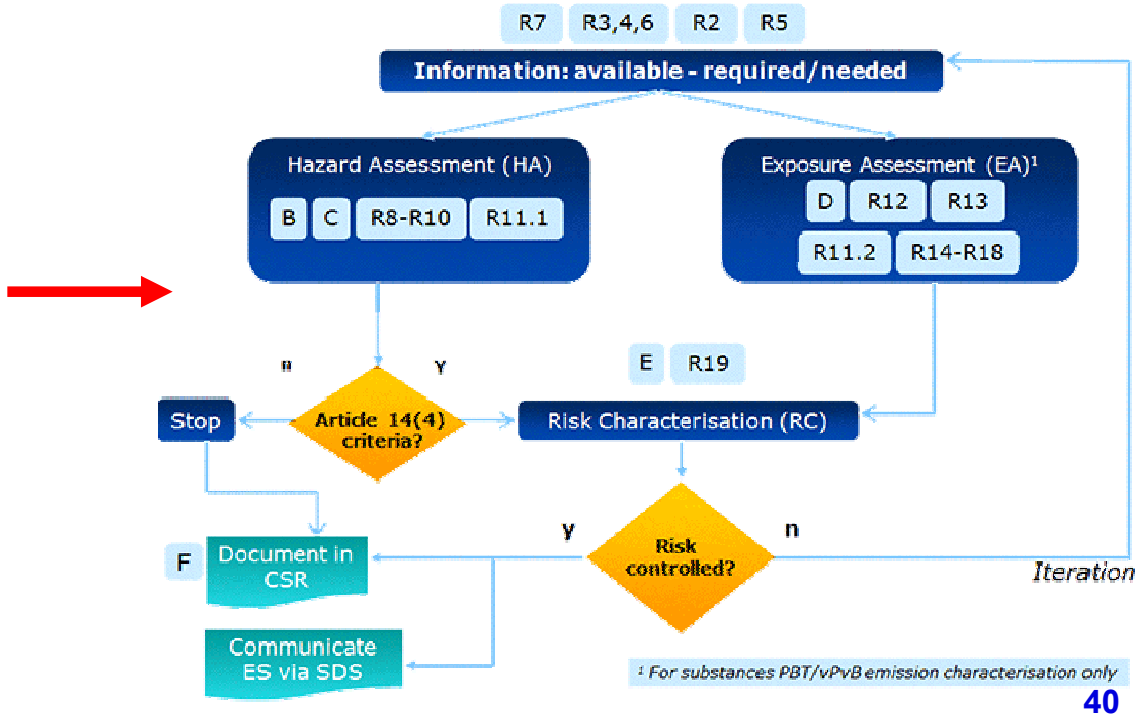
C.1.	ACUTE TOXICITY FOR FISH
C.2.	*DAPHNIA* SP. ACUTE IMMOBILISATION TEST
C.3.	FRESHWATER ALGA AND CYANOBACTERIA, GROWTH INHIBITION TEST
C.4.	DETERMINATION OF 'READY' BIODEGRADABILITY
PART I.	GENERAL CONSIDERATIONS
PART II.	DOC DIE-AWAY TEST (Method C.4-A)
PART III.	MODIFIED OECD SCREENING TEST (Method C.4-B)
PART IV.	CO_2 EVOLUTION TEST (Method C.4-C)
PART V.	MANOMETRIC RESPIROMETRY TEST (Method C.4-D)
PART VI.	CLOSED BOTTLE TEST (Method C.4-E)
PART VII.	M.I.T.I. TEST (Method C.4-F)
C.5.	DEGRADATION — BIOCHEMICAL OXYGEN DEMAND
C.6.	DEGRADATION — CHEMICAL OXYGEN DEMAND
C.7.	DEGRADATION — ABIOTIC DEGRADATION: HYDROLYSIS AS A FUNCTION OF PH
C.8.	TOXICITY FOR EARTHWORMS
C.9.	BIODEGRADATION — ZAHN-WELLENS TEST
C.10.	SIMULATION TESTAEROBIC SEWAGE TREATMENT: C.10-A: ACTIVATED SLUDGE UNITS — C.10-B: BIOFILMS
C.11.	ACTIVATED SLUDGE, RESPIRATION INHIBITION TEST (CARBON AND AMMONIUM OXIDATION)
C.12.	BIODEGRADATION — MODIFIED SCAS TEST
C.13.	BIOACCUMULATION IN FISH: AQUEOUS AND DIETARY EXPOSURE
C.14.	FISH JUVENILE GROWTH TEST
C.15.	FISH, SHORT-TERM TOXICITY TEST ON *EMBRYO* AND SAC-FRY STAGES
C.16.	HONEYBEES — ACUTE ORAL TOXICITY TEST
C.17.	HONEYBEES — ACUTE CONTACT TOXICITY TEST
C.18.	ADSORPTION/DESORPTION USING A BATCH EQUILIBRIUM METHOD
C.19.	ESTIMATION OF THE ADSORPTION COEFFICIENT (K_{OC}) ON SOIL AND ON SEWAGE SLUDGE USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)
C.20.	*DAPHNIA MAGNA* REPRODUCTION TEST
C.21.	SOIL MICROORGANISMS: NITROGEN TRANSFORMATION TEST
C.22.	SOIL MICROORGANISMS: CARBON TRANSFORMATION TEST
C.23.	AEROBIC AND ANAEROBIC TRANSFORMATION IN SOIL
C.24.	AEROBIC AND ANAEROBIC TRANSFORMATION IN AQUATIC SEDIMENT SYSTEMS
C.25.	AEROBIC MINERALISATION IN SURFACE WATER — SIMULATION BIODEGRADATION TEST
C.26.	LEMNA SPECIES GROWTH INHIBITION TEST
C.27.	SEDIMENT-WATER CHIRONOMID TOXICITY TEST USING SPIKED SEDIMENT
C.28.	SEDIMENT-WATER CHIRONOMID TOXICITY TEST USING SPIKED WATER
C.29.	READY BIODEGRADABILITY — CO_2 IN SEALED VESSELS (HEADSPACE TEST)
C.30.	BIOACCUMULATION IN TERRESTRIAL OLIGOCHAETES
C.31.	TERRESTRIAL PLANT TEST: SEEDLING EMERGENCE AND SEEDLING GROWTH TEST
C.32.	ENCHYTRAEID REPRODUCTION TEST
C.33.	EARTHWORM REPRODUCTION TEST (*EISENIA FETIDA/EISENIA ANDREI*)
C.34.	DETERMINATION OF THE INHIBITION OF THE ACTIVITY OF ANAEROBIC BACTERIA — REDUCTION OF GAS PRODUCTION FROM ANAEROBICALLY DIGESTING (SEWAGE) SLUDGE
C.35.	SEDIMENT-WATER *LUMBRICULUS* TOXICITY TEST USING SPIKED SEDIMENT
C.36.	PREDATORY MITE (*HYPOASPIS (GEOLAELAPS) ACULEIFER*) REPRODUCTION TEST IN SOIL
C.37.	21-DAY FISH ASSAY: A SHORT-TERM SCREENING FOR OESTROGENIC AND ANDROGENIC ACTIVITY, AND AROMATASE INHIBITION
C.38.	THE AMPHIBIAN METAMORPHOSIS ASSAY
C.39.	COLLEMBOLAN REPRODUCTION TEST IN SOIL
C.40.	SEDIMENT-WATER CHIRONOMID LIFE-CYCLE TOXICITY TEST USING SPIKED WATER OR SPIKED SEDIMENT
C.41.	FISH SEXUAL DEVELOPMENT TEST
C.42.	BIODEGRADABILITY IN SEAWATER
C.43.	ANAEROBIC BIODEGRADABILITY OF ORGANIC SUBSTANCES IN DIGESTED SLUDGE: BY MEASUREMENT OF GAS PRODUCTION
C.44.	LEACHING IN SOIL COLUMNS
C.45.	ESTIMATION OF EMISSIONS FROM PRESERVATIVE — TREATED WOOD TO THE ENVIRONMENT: LABORATORY METHOD FOR WOODEN COMMODITIES THAT ARE NOT COVERED AND ARE IN CONTACT WITH FRESH WATER OR SEAWATER
C.46.	BIOACCUMULATION IN SEDIMENT-DWELLING BENTHIC OLIGOCHAETES
C.47.	FISH, EARLY-LIFE STAGE TOXICITY TEST
C.48.	FISH SHORT TERM REPRODUCTION ASSAY
C.49.	FISH EMBRYO ACUTE TOXICITY (FET) TEST
C.50.	SEDIMENT-FREE *MYRIOPHYLLUM SPICATUM* TOXICITY TEST
C.51.	WATER-SEDIMENT *MYRIOPHYLLUM SPICATUM* TOXICITY TEST
C.52.	MEDAKA EXTENDED ONE GENERATION REPRODUCTION TEST (MEOGRT)
C.53.	THE LARVAL AMPHIBIAN GROWTH AND DEVELOPMENT ASSAY (LAGDA)

REACH

- **chemical safety assessment ~ risk assessment principle = combination of HAZARD and EXPOSURE**
- detailed guidelines for each phase

https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment





REACH

Environmental hazard assessment

- **PNEC (predicted no effect concentration)** for each environmental compartment
- concentration below which adverse effects on ecosystems will not occur
- derived on basis of available information on toxicity to species from relevant environments, i.e. toxicity test endpoints (LC50s or NOECs/EC10s), using appropriate **assessment factors (AF)** to extrapolate from single-species laboratory data to a multi-species ecosystem, to address:
  - intra- and inter-laboratory variation of toxicity data
  - intra- and inter-species variations (biological variance)
  - short-term to long-term toxicity extrapolation
  - laboratory data to field impact extrapolation
  - ...

REACH

Environmental hazard assessment

- **PNEC determination**

$$PNEC_{comp} = \frac{Min\{EC_{comp}\}}{AF}$$

Input

Parameter	Description	Source
Min{EC_{comp}}	The lowest valid effect concentration for organisms from the compartment, i.e. EC50 or LC50 for short-term toxicity or EC10/NOEC for long-term toxicity, typically given in [mg/L] or [mg/kg]	Technical Dossier [cf. Art. 10 (a) (vi) and (vii)]
AF	Assessment factor, the size of which depends on the type and amount of toxicity information available	Chapter R.10.3.1

Output

Parameter	Description	Use
$PNEC_{comp}$	Predicted No-Effect-Concentration for the compartment in question, typically given in [mg/L] or [mg/kg]	Risk assessment

ECHA (2011): Guidance on information requirements and chemical safety assessment Part B: Hazard assessment. https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

Table R.10-4 Assessment factors to derive a $PNEC_{aquatic}$

Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels (fish, invertebrates (preferred Daphnia) and algae)	1000 [a]
One long-term EC10 or NOEC (either fish or Daphnia)	100 [b]
Two long-term results (e.g. EC10 or NOECs) from species representing two trophic levels (fish and/or Daphnia and/or algae)	50 [c]
Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10 [d]
Species sensitivity distribution (SSD) method	5-1 (to be fully justified case by case) [e]
Field data or model ecosystems	Reviewed on a case by case basis [f]

ECHA (2008): Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose [concentration]-response for environment. https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

REACH

Environmental hazard assessment

- **PNEC determination**

Example:

A dossier for a substance manufactured in quantities between 10 and 100 tonnes (Annex VIII requirements) has the following ecotoxicity data

Algae: *Scenedesmus subspicatus* EC50 (72 hours) = 10 mg/L

Invertebrates: *Daphnia magna* EC50 (48 hours) = 1 mg/L

Fish: *Pimephales promelas* EC50 (96 hours) = 0.8 mg/L

In this situation only short-term ecotoxicity data are available. The most sensitive trophic level is the fish with an EC50(96 hours) = 0.8 mg/L (=min{EC_{hwater}}).

According to Section R.10.3.1.2 the assessment factor (AF) to use when only short term toxicity data are available on the three trophic levels is 1000.

The PNEC*water* = 0.8 / 1000 = 0.0008 mg/L = 0.8µg/L

ECHA (2011): Guidance on information requirements and chemical safety assessment Part B: Hazard assessment. https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

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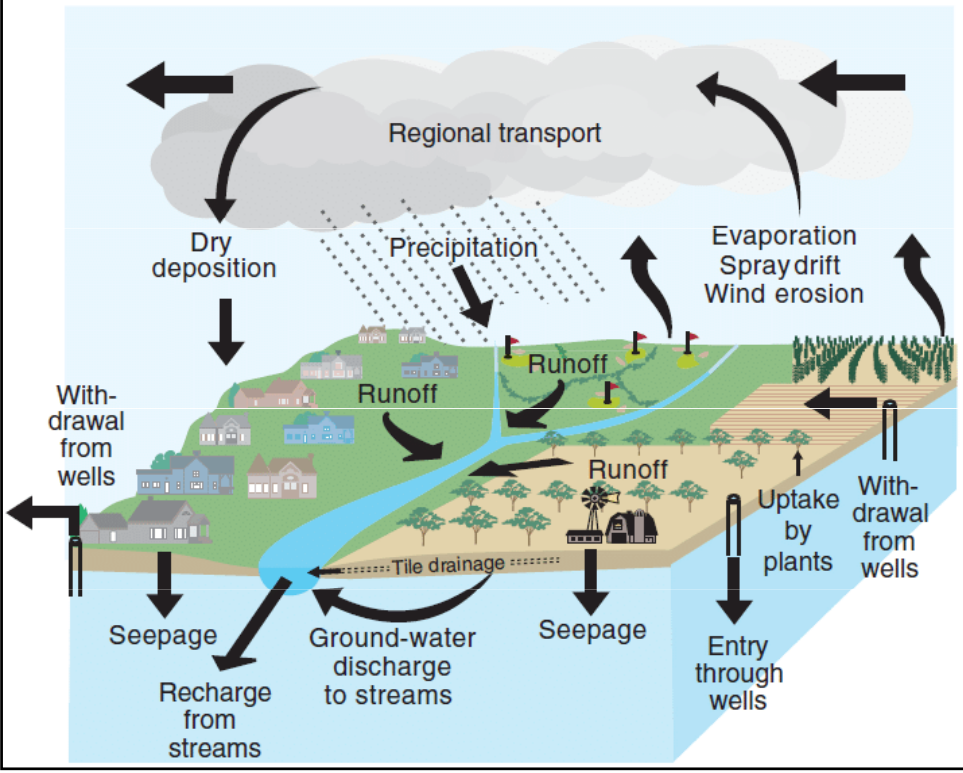
Exposure assessment

- **PEC (predicted environmental concentration)** for each environmental compartment
- models based on production of chemicals,



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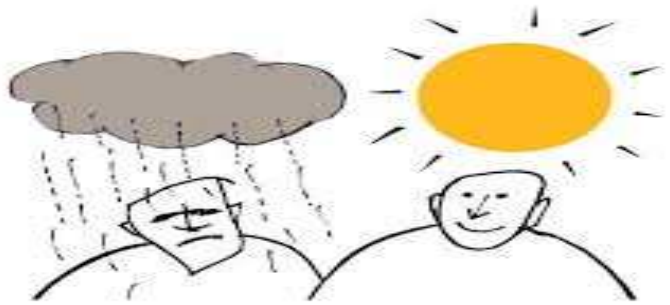
Environmental risk characterization

Local	Regional
Water: $PEClocal_{water}/PNEC_{water}$	Water: $PECregional_{water}/PNEC_{water}$
Sediment: $PEClocal_{sediment}/PNEC_{sediment}$	Sediment: $PECregional_{sediment}/PNECs_{ediment}$
Soil: $PEClocal_{soil}/PNEC_{soil}$	Soil: $PECregional_{agr.soil}/PNEC_{soil}$
RMicroorganisms: $PEC_{stp}/PNEC_{microorganisms}$	
Predators, fish eating $(0.5 \cdot PEClocal,oral_{fish} + 0.5 \cdot PECregional,oral_{fish})/PNECoral$	
Predators, worm-eating $(0.5 \cdot PEClocal,oral_{worm} + 0.5 \cdot PECregional,oral_{worm})/PNECoral$	

$$HQ = \frac{PEC}{PNEC}$$



PEC > PNEC (HQ>1)

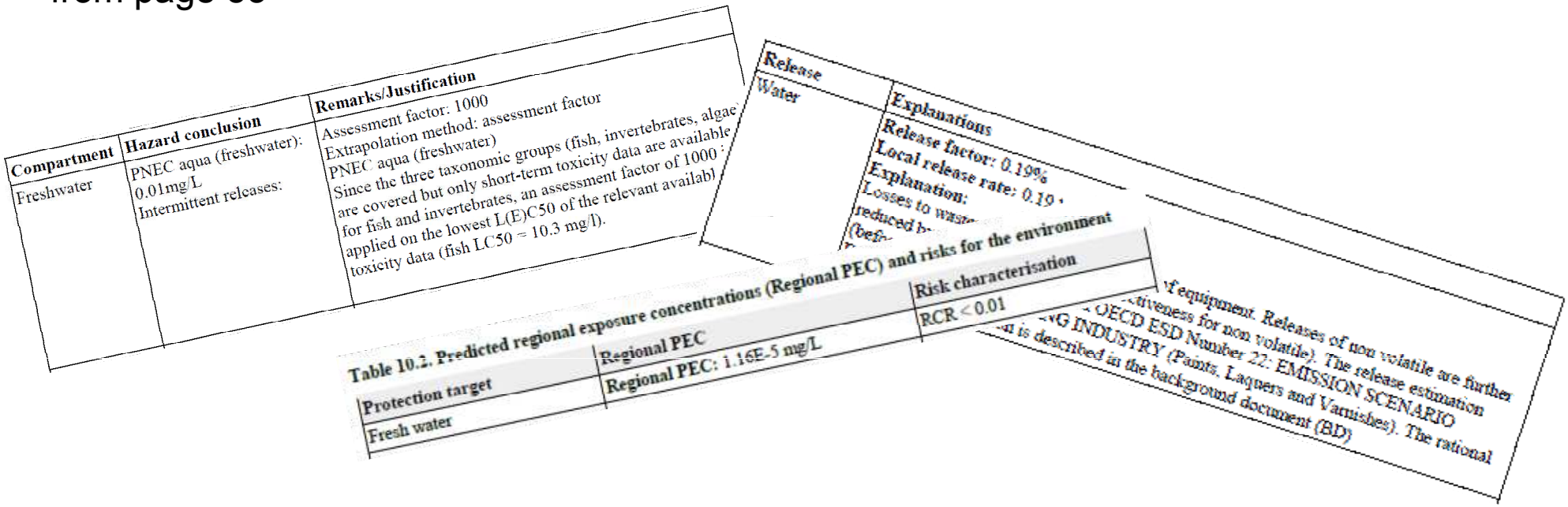
PEC < PNEC (HQ<1)

REACH

Example CSR report

https://echa.europa.eu/support/practical-examples-of-chemical-safety-reports

from page 53



Compartment	Hazard conclusion	Remarks/Justification
Freshwater	PNEC aqua (freshwater): 0.01mg/L Intermittent releases:	Assessment factor: 1000 Extrapolation method: assessment factor PNEC aqua (freshwater) Since the three taxonomic groups (fish, invertebrates, algae) are covered but only short-term toxicity data are available for fish and invertebrates, an assessment factor of 1000 is applied on the lowest L(E)C50 of the relevant availab toxicity data (fish LC50 = 10.3 mg/l).

Release	Explanations
Water	Release factor: 0.19% Local release rate: 0.19 Explanation: Losses to was reduced b (befi

Table 10.2. Predicted regional exposure concentrations (Regional PEC) and risks for the environment

Protection target	Regional PEC	Risk characterisation
Fresh water	Regional PEC: 1.16E-5 mg/L	RCR < 0.01

f equipment. Releases of non volatile are further
tiveness for non volatile). The release estimation
OECD ESD Number 22: EMISSION SCENARIO
G INDUSTRY (Paints, Laquers and Varnishes). The rational
is described in the background document (BD)

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- some data available in EC inventory and IUCLID 6
- but not full Chemical Safety Reports
- example: https://echa.europa.eu/substance-information/-/substanceinfo/100.100.840

https://iuclid6.echa.europa.eu/project-iuclid-6

ECHA > Information on Chemicals > EC Inventory

EC Inventory https://echa.europa.eu/cs/information-on-chemicals/ec-inventory

The EC inventory published below is a copy as received from the JRC in 2008 on the founding of ECHA. It is comprised of the following lists:

- **EINECS** (European INventory of Existing Commercial chemical Substances) as published in O.J. C 146A, 15.6.1990. EINECS is an inventory of substances that were deemed to be on the European Community market between 1 January 1971 and 18 September 1981. EINECS was drawn up by the European Commission in the application of Article 13 of Directive 67/548/EEC, as amended by Directive 79/831/EEC, and in accordance with the detailed provisions of Commission Decision 81/437/EEC. Substances listed in EINECS are considered phase-in substances under the REACH Regulation.
- **ELINCS** (European LIst of Notified Chemical Substances) in support of Directive 92/32/EEC, the 7th amendment to Directive 67/548/EEC. ELINCS lists those substances which were notified under Directive 67/548/EEC, the Dangerous Substances Directive Notification of New Substances (NONS) that became commercially available after 18 September 1981.
- **NLP** (No-Longer Polymers). The definition of polymers was changed in April 1992 by Council Directive 92/32/EEC amending Directive 67/548/EEC, with the result that substances previously considered to be polymers were no longer excluded from regulation. Thus the No-longer Polymers (NLP) list was drawn up, consisting of such substances that were commercially available between 18 September 1981 and 31 October 1993.

Last updated 11 August 2017. Database contains 106211 unique substances/entries.

Filter the list

Page 1 of 2,125 | 50 Items per Page | Showing 1 - 50 of 106,211 results. | ← First | Previous | Next | Last →

Name	EC no.	CAS no.	Molecular Formula	Description
"mercurous oxide"	239-934-0	15829-53-5	Hg2O	
((2-ethyl-1-oxohexyl)oxy)-(1-phenyl-1,3-decanedionyl)dioctyl stannane RHODORSIL ACCELERATEUR 2025	422-920-5	-		RHODORSIL ACCELERATEUR 2025
((4-phenylbutyl)hydroxyphosphoryl)acetic acid	412-170-7	-		SQ 26999

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- for chemicals, producers and importers must provide information about risks related to substance use, based on **Regulation No 1272/2008, on the classification, labelling and packaging of substances and mixtures (CLP Regulation)** https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02008R1272-20201114
- information on risks is provided in agreement with **Globally Harmonised System of Classification and Labelling of Chemicals (https://unece.org/about-ghs)**
- **among plenty of other data and properties, ecotoxicity is evaluated**

GHS

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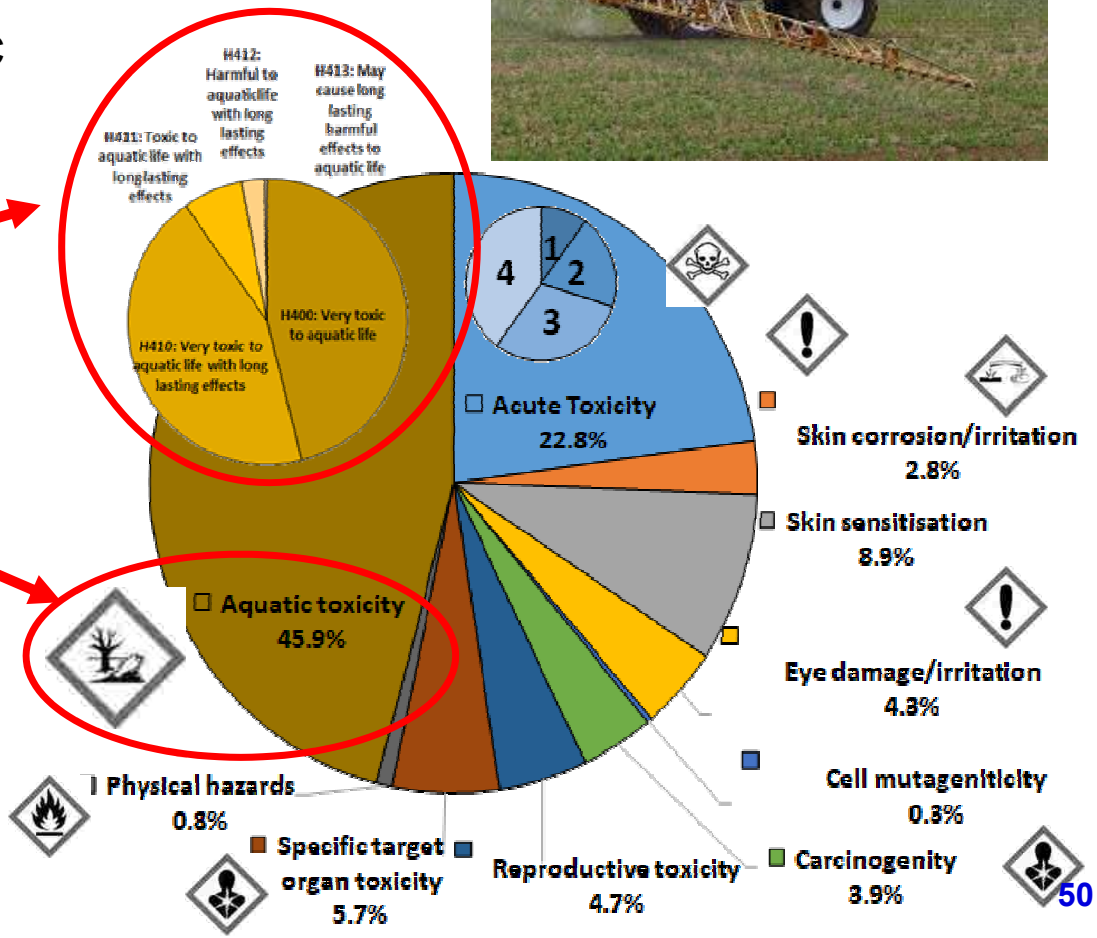
Example 3
Pesticides - Plant protection products (PPP) – in EU

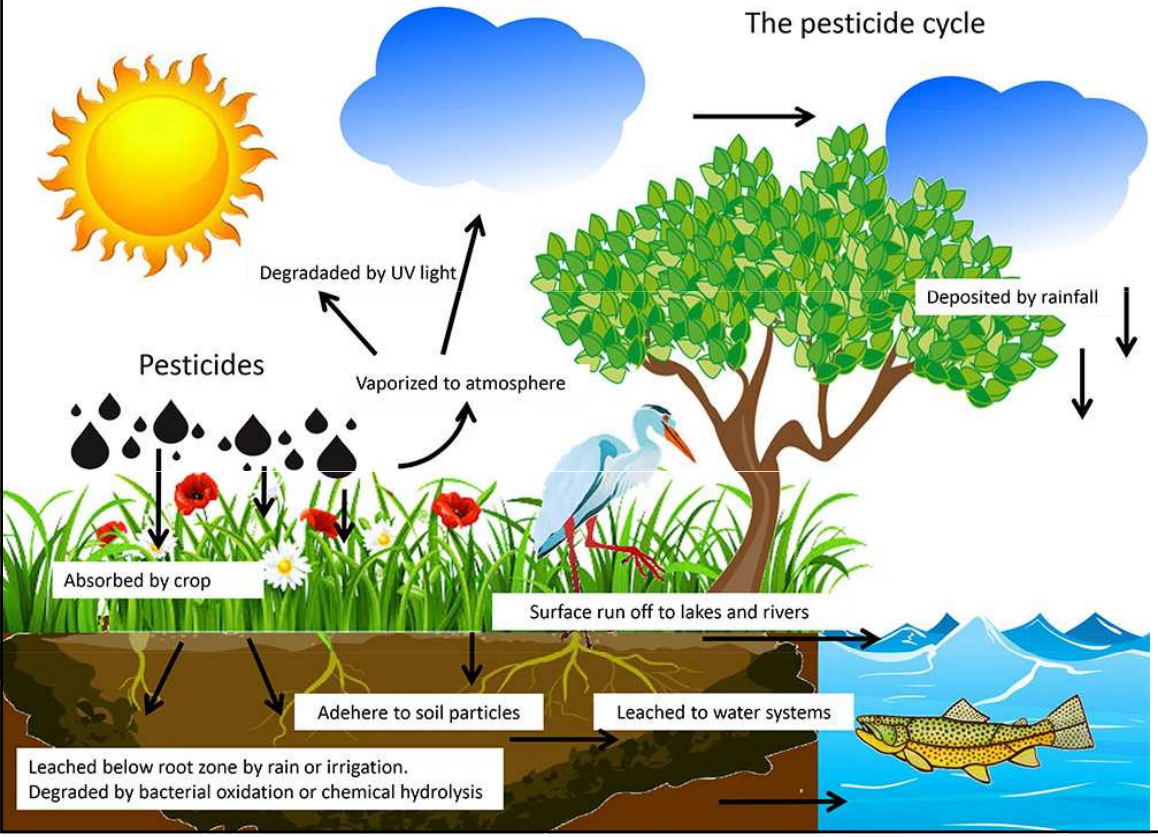
Plant protection products (PPP)

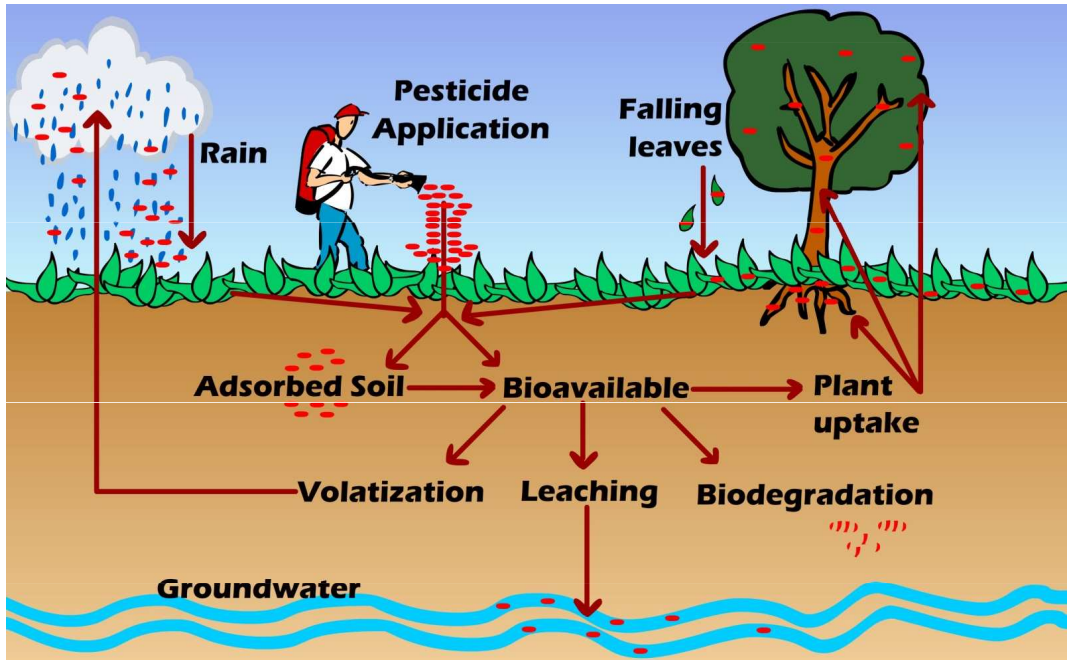
- **pesticides are very hazardous chemicals!**
- Hofman analysis 11/2018: **204 / 441 approved AS have some classification according to EC 1272/2008 (CLP) -** majority AS have 3 or more (max 9 – e.g. Spiroxamin), in total there are 723 hazard statements found for all approved AS
  - 12 acute toxicity 1
  - 28 carcinogenicity 2
  - **153 acute aq. tox. 1**
  - 11 reprod. tox. 1B



Plant protection products (PPP)

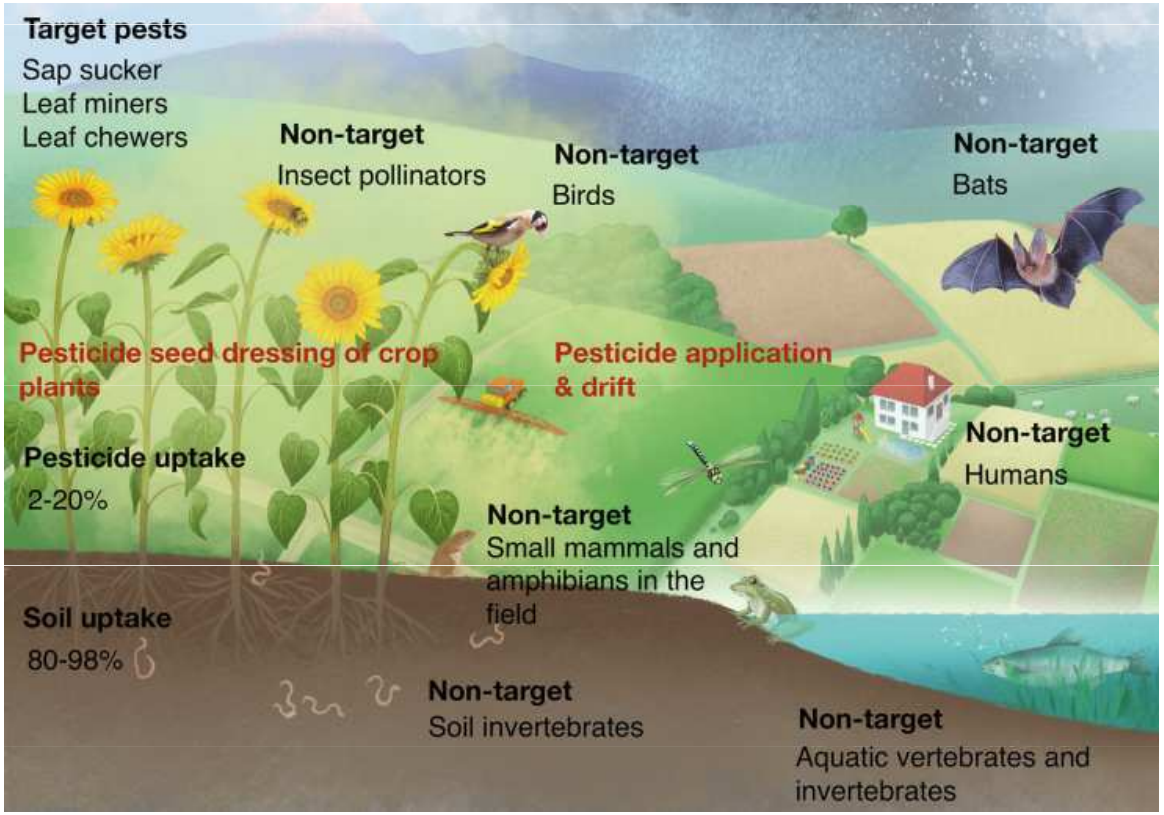
- ecotoxicity assessment is related to the expected behavior and impacts after PPP application
- lot of non-target biota under risk of undesired negative impact

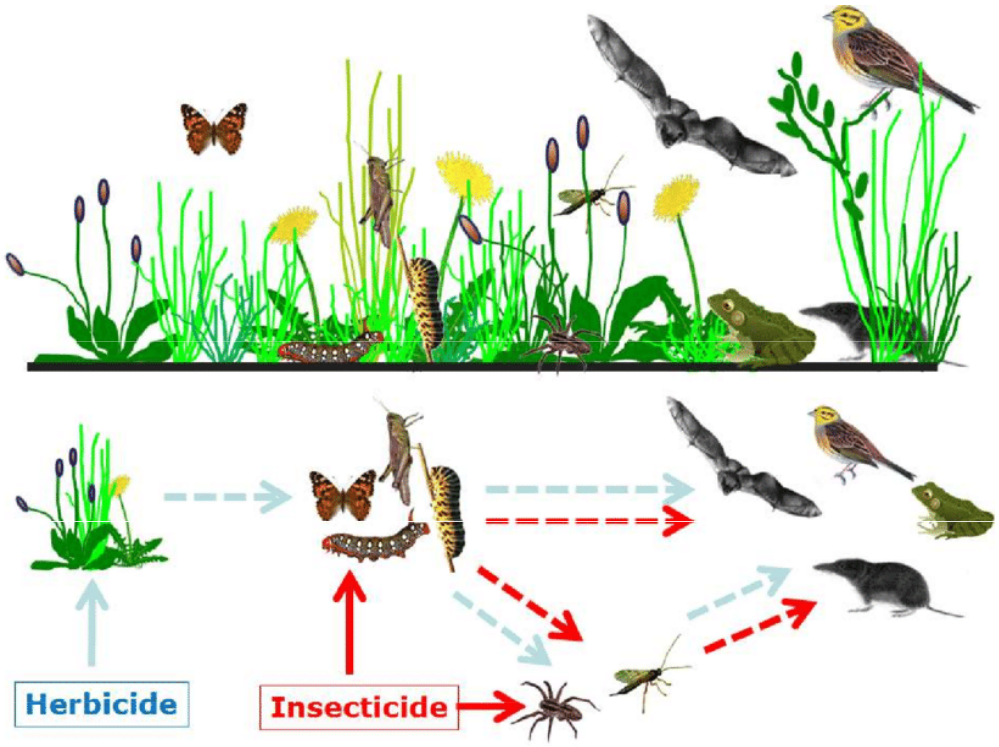




Plant protection products (PPP)

- lot of non-target biota under risk of undesired negative impact, including indirect effects





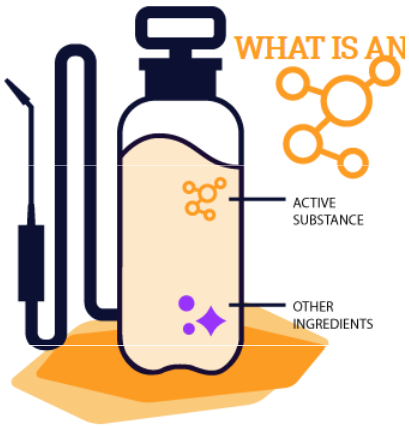
Plant protection products (PPP)

- https://ec.europa.eu/food/plant/pesticides_en
- www.efsa.europa.eu/en/topics/topic/pesticides

- in EU, EFSA is responsible for the authorization of PPP
- **Regulation 1107/2009, concerning the placing of plant protection products on the market**
  https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02009R1107-20191214
- **Regulation 546/2011, uniform principles for evaluation and authorisation of PPP**
  https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02011R0546-20180524

- **overview of whole process:**
  https://www.efsa.europa.eu/en/interactive-pages/pesticides-authorisation/PesticidesAuthorisation
  https://ec.europa.eu/assets/sante/food/plants/pesticides/lop/index.html

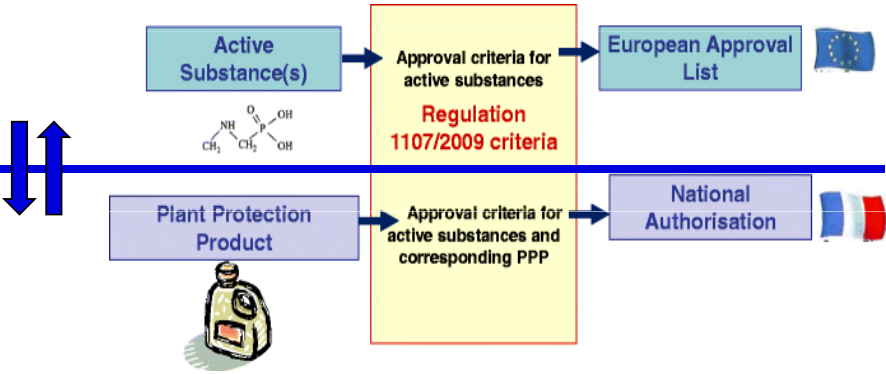
Plant protection products (PPP)



- the whole system is divided to:

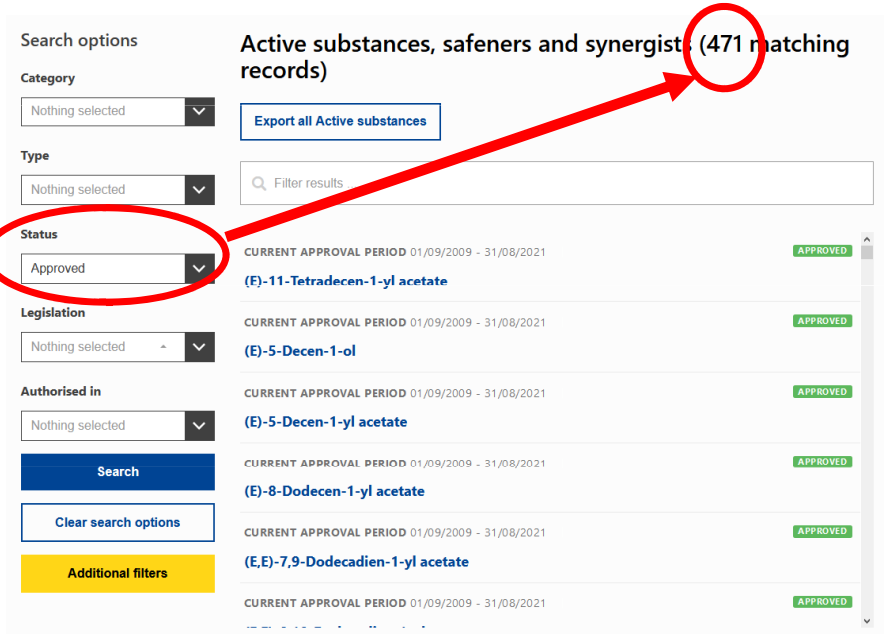
- **active substances approval** - done for whole EU
  a.s. – substance having action against harmful organisms

- **authorization of PPP** – done in member states (zones)
  mix of a.s. and other components: safeners (eliminate or reduce phytotoxic effects of PPP), synergists (enhance a.s. effect), co-formulants (other components), adjuvants (added to PPP by end-user)

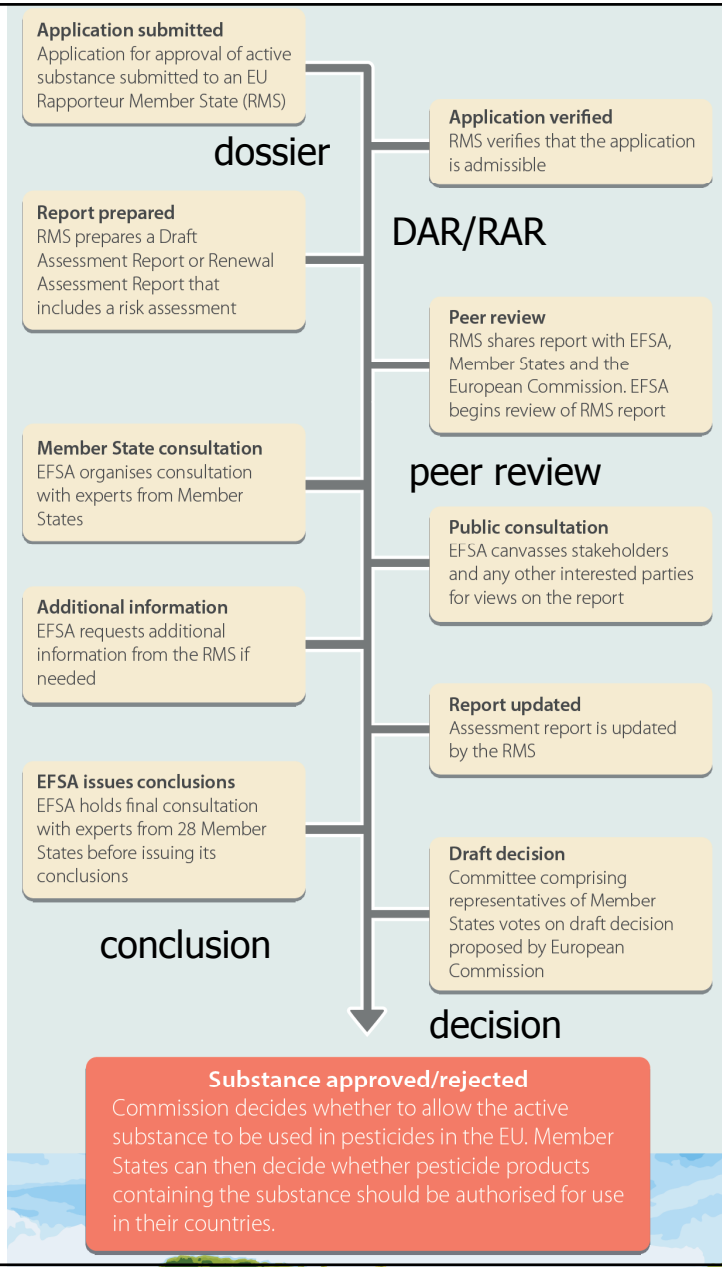


Approval of a.s.

- Regulation 283/2013, setting out the **data requirements for active substances** https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02013R0283-20141117
- Regulation 540/2011, **list of approved active substances** https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02011R0540-20201126
- EU pesticide database **https://ec.europa.eu/food/plant/pesticides/eu-pesticides-db_en**



- its decided if a.s. is approved or not, what conditions and labelling (CLP) and also limits for residues are set (MRL)
- its valid for 10 year and then re-assessment



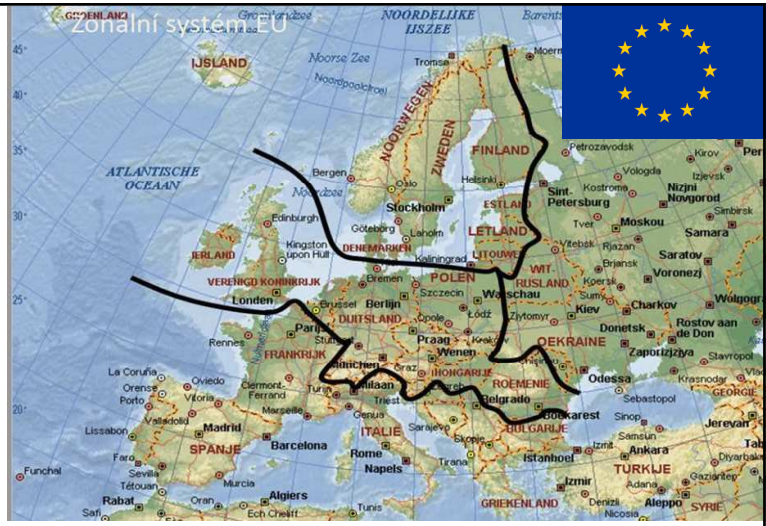
Approval of a.s.

- Regulation 283/2013 defines what data, **including ecotoxicological**, are required for approval of active substance
- ANNEX section 8
- some details provided on the bioassays performance, but more in specific guideline documents

8.1.	**Effects on birds and other terrestrial vertebrates**	8.3.	**Effect on arthropods**
8.1.1.	Effects on **birds**	8.3.1.	Effects on **bees**
8.1.1.1.	Acute oral toxicity to birds	8.3.1.1.	Acute toxicity to bees
8.1.1.2.	Short-term dietary toxicity to birds	8.3.1.1.1.	Acute oral toxicity
8.1.1.3.	Sub-chronic and reproductive toxicity to birds	8.3.1.1.2.	Acute contact toxicity
8.1.2.	Effects on terrestrial vertebrates other than birds	8.3.1.2.	Chronic toxicity to bees
8.1.2.1.	**Acute** oral toxicity to **mammals**	8.3.1.3.	Effects on honeybee development and other honeybee life stages
8.1.2.2.	**Long-term** and reproductive toxicity to **mammals**	8.3.1.4.	Sub-lethal effects
8.1.3.	Active substance bioconcentration in prey of birds and mammals	8.3.2.	Effects on non-target arthropods other than bees
8.1.4.	Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)	8.3.2.1.	Effects on **Aphidius** rhopalosiphi
8.1.5.	Endocrine disrupting properties	8.3.2.2.	Effects on **Typhlodromus** pyri
8.2.	**Effects on aquatic organisms**	8.4.	**Effects on non-target soil meso- and macrofauna**
8.2.1.	**Acute** toxicity to **fish**	8.4.1.	**Earthworm** — sub-lethal effects
8.2.2.	**Long-term and chronic** toxicity to **fish**	8.4.2.	Effects on non-target soil meso- and macrofauna (other than earthworms)
8.2.2.1.	Fish early life stage toxicity test	8.4.2.1.	Species level testing
8.2.2.2.	Fish full life cycle test	8.5.	**Effects on soil nitrogen transformation**
8.2.2.3.	Bioconcentration in fish	8.6.	**Effects on terrestrial non-target higher plants**
8.2.3.	Endocrine disrupting properties	8.6.1.	Summary of screening data
8.2.4.	Acute toxicity to aquatic invertebrates	8.6.2.	Testing on non-target plants
8.2.4.1.	**Acute** toxicity to **Daphnia magna**	8.7.	Effects on other terrestrial organisms (flora and fauna)
8.2.4.2.	Acute toxicity to an additional aquatic invertebrate species	8.8.	**Effects on biological methods for sewage treatment**
8.2.5.	Long-term and chronic toxicity to aquatic invertebrates	8.9.	Monitoring data
8.2.5.1.	**Reproductive** and development toxicity to **Daphnia magna**		
8.2.5.2.	Reproductive and development toxicity to an additional aquatic invertebrate species		
8.2.5.3.	Development and emergence in **Chironomus riparius**		
8.2.5.4.	Sediment dwelling organisms		
8.2.6.	Effects on algal growth		
8.2.6.1.	Effects on growth of green **algae**		
8.2.6.2.	Effects on growth of an additional algal species		
8.2.7.	Effects on aquatic **macrophytes**		

Authorization of PPP

- Regulation 284/2013, setting out the **data requirements for PPP**
  https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02013R0284-20150917
- example of PPP database from CR
  http://eagri.cz/public/app/eagriapp/POR/Vyhledavani.aspx?type=0&vyhledat=A&stamp=1609840149173



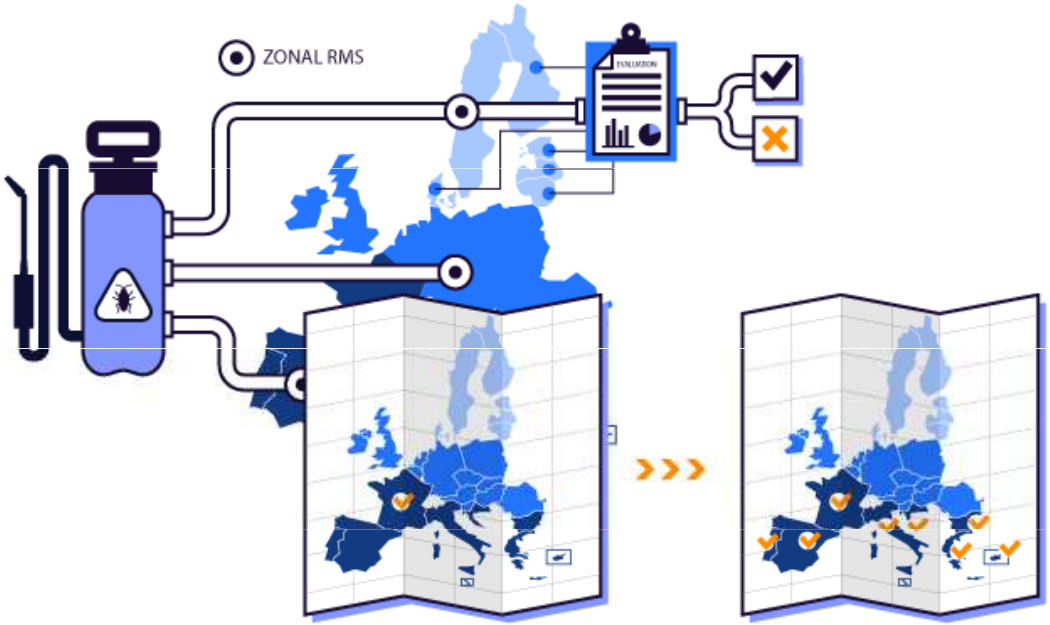
Product Search

Kritéria vyhledávání:
▪ **Aktuální stav rozhodnutí**: Platné rozhodnutí + Do spotřebování zásob

Počet nalezených záznamů: **3509**

[back] [remove filters] [export into the table]

Product Name	Reg. Number	Authorisation Holder	Field of Use	Active Substance	Product Expiry Date	Sale and Distribution of Existing Stocks	Disposal, Storage and Use of Existing Stocks	Current Status of Approval	Remark	
AA-SULPHUR 80 WG	4985-1	CIECH Sarzyna Spółka Akcyjna	F	Síra (*Sulphur*)	31.12.2021	31.12.2021	31.12.2021	Platné rozhodnutí		
ABAM	3978-6D/8	CMI Limited	I	Abamektin (*Abamectin*)	30.4.2022	30.4.2022	30.4.2022	Platné rozhodnutí	2	
Abamectin-Q 18 EC	3978-6D/9	Q-CHEM NV	I	Abamektin (*Abamectin*)	30.4.2022	30.4.2022	30.4.2022	Platné rozhodnutí	2	
Abilis Ultra	3975-12	Bayer AG	F	Tebukonazol (*Tebuconazole*)	31.8.2022	31.8.2022	31.8.2022	Platné rozhodnutí		
Accent 75 WG	4596-0	DuPont CZ s.r.o.	H	Nikosulfuron (*Nicosulfuron*)	31.12.2022	31.12.2022	31.12.2022	Platné rozhodnutí		
Accord WG	4649-0D/2	AUVERONE s.r.o.	F	Folpet (*Folpet*), Iprovalikarb (*Iprovalicarb*)	31.7.2021	31.7.2021	31.7.2021	Platné rozhodnutí	2	
Accurate Delta	4828-2	Nufarm GmbH and Co KG	H	Diflufenikan (*Diflufenican*), Metsulfuron-methyl (*Metsulfuron-methyl*)	31.12.2021	31.12.2021	31.12.2021	Platné rozhodnutí		
Accurate Extra	5233-1	Nufarm GmbH and Co KG	H	Metsulfuron-methyl (*Metsulfuron-methyl*), Thifensulfuron-methyl (*Thifensulfuron-methyl*)	31.3.2023	31.3.2023	31.3.2023	Platné rozhodnutí		
Aceptir 200	5385-1	INNVIGO Sp. z o.o.	I	Acetamiprid	30.4.2022	30.4.2022	30.4.2022	Platné		



Authorization of PPP

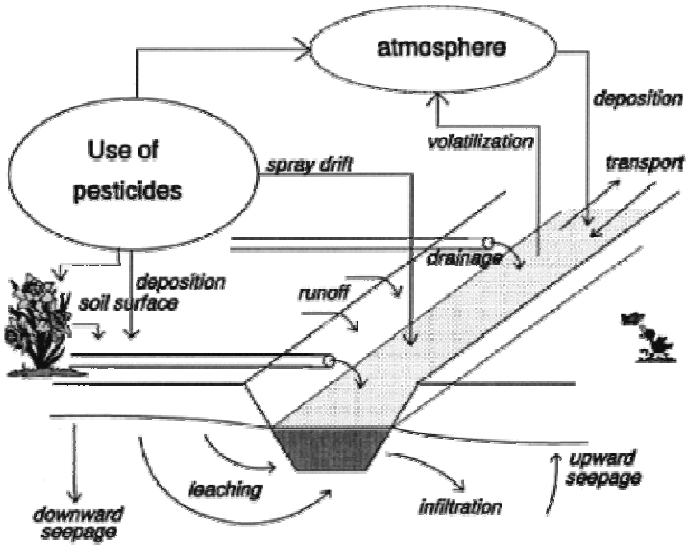
- Regulation 284/2013 defines what data, **including ecotoxicological**, are required for PPP authorization
- ANNEX section 10
- some details provided on the bioassays performance, but more in specific guideline documents
- testing necessary where PPP toxicity cannot be predicted on the basis of data on AS
- aim = to demonstrate PPP is more toxic than AS (bridging studies or limit test may be sufficient); if yes, definitive testing is required

10.1.	**Effects on birds and other terrestrial vertebrates**	10.4.	**Effects on non-target soil meso- and macrofauna**
10.1.1.	Effects on birds	10.4.1.	Earthworms
10.1.1.1.	Acute oral toxicity to birds	10.4.1.1.	Earthworms — sub-lethal effects
10.1.1.2.	Higher tier data on birds	10.4.1.2.	Earthworms — field studies
10.1.2.	Effects on terrestrial vertebrates other than birds	10.4.2.	Effects on non-target soil meso- and macrofauna (other than earthworms)
10.1.2.1.	Acute oral toxicity to mammals	10.4.2.1.	Species level testing
10.1.2.2.	Higher tier data on mammals	10.4.2.2.	Higher tier testing
10.1.3.	Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)	10.5.	**Effects on soil nitrogen transformation**
10.2.	**Effects on aquatic organisms**	10.6.	**Effects on terrestrial non-target higher plants**
10.2.1.	Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes	10.6.1.	Summary of screening data
10.2.2.	Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms	10.6.2.	Testing on non-target plants
10.2.3.	Further testing on aquatic organisms	10.6.3.	Extended laboratory studies on non-target plants
10.3.	Effects on arthropods	10.6.4.	Semi-field and field tests on non-target plants
10.3.1.	**Effects on bees**	10.7.	Effects on other terrestrial organisms (flora and fauna)
10.3.1.1.	Acute toxicity to bees	10.8.	Monitoring data
10.3.1.1.1.	Acute oral toxicity		
10.3.1.1.2.	Acute contact toxicity		
10.3.1.2.	Chronic toxicity to bees		
10.3.1.3.	Effects on honey bee development and other honey bee life stages		
10.3.1.4.	Sub-lethal effects		
10.3.1.5.	Cage and tunnel tests		
10.3.1.6.	Field tests with honeybees		
10.3.2.	**Effects on non-target arthropods other than bees**		
10.3.2.1.	Standard laboratory testing for non-target arthropods		
10.3.2.2.	Extended laboratory testing, aged residue studies with non-target arthropods		
10.3.2.3.	Semi-field studies with non-target arthropods		
10.3.2.4.	Field studies with non-target arthropods		
10.3.2.5.	Other routes of exposure for non-target arthropods		

PPP exposure assessment

exposure is expressed by different ways:

- directly as application rate (e.g. g a.s. / ha)
  - initial, maximum or modifications
- as concentrations in various compartments
  - **Predicted Exposure Concentration – PEC (PECs, PECsw ...)**
  - predicted by different **environmental fate models**
    e.g. https://esdac.jrc.ec.europa.eu/projects/focus-dg-sante
    e.g. https://www.pesticidemodels.eu/swash/home
    = models of **transport and behavior** of PPP in the environment (drift, sorption, degradation, mobility, accumulation ...) in **time**
- dietary daily intake (DDD) – for birds and mammals



Simplified exposure:

$DDD_{acute} = SV_{90} * MAF_{90} * \text{single appl. rate}$

SV = FIR/bw*RUD*DF

PT; PD = set to 1

DDD = (FIR/b.w.) * RUD * DF *MAF * PT * PD * single appl. rate

where

SV = Shortcut Value (→ see Appendix A of EFSA GD 2009)
FIR/bw = Food intake rate / body weight (→ see Appendix G/L of EFSA GD 2009)
RUD = Residue Unit Dose
MAF = Multiple Application Factor (→ see also Appendix H of EFSA GD 2009)
DF = Deposition Factor (→ see Appendix E of EFSA GD 2009)
PD = Portion of Diet (→ see Appendix Q of EFSA GD 2009)
PT = Portion of Time (→ see Appendix P of EFSA GD 2009)

PPP ecotoxicity and risk assessment

- assessment of (eco)toxicity and acute / short-term / long-term risks for:
  - birds and other terrestrial vertebrates
  - aquatic organisms (fish, inventebrates, algae, plants, sediment organisms)
  - bees
  - non-target arthropods
  - earthworms
  - soil macro- and mesofauna (other than earthworms)
  - soil microorganisms (C mineralization, N mineralization)
  - non target plants
  - biological methods of sewage treatment

PPP ecotoxicity and risk assessment

- fundamental principle: **risk assessment = combining effects and exposures**
  - most often as **TER** = **toxicity/exposure ratio = ratio of effect endpoint and estimated exposure**
  - but for bees or non-target arthtropods, hazard quotient (HQ) - ratio between exposure and toxicity (usually in units of g a.s. / ha)
- used endpoints (LD50, NOEL, LC50, EC50, NOEC ... acute, short-, or long-term) and units (e.g. in body or in environment, initial, long- or short-term ...) depend on the specific bioassay and results
- **the most sensitive organism** used in the tests has key influence on the final decisions

PPP ecotoxicity and risk assessment

- TER is compared to **trigger** (= Assessment Factor = Safety Factor)
- these are laid down in Regulation 546/2011 and Guidance Documents
- example for birds and mammals (tier 1):

$$TER = \frac{\text{toxicity value}}{\text{exposure}}$$

$$TER_{acute} = \frac{LD_{50}}{DDD_a} \qquad \text{Trigger*: } TER \geq 10$$

$$TER_{longterm} = \frac{NOEL}{DDD_{lt}} \qquad TER \geq 5$$

- example for aquatic organisms (tier 1):

Group and timescale	Trigger
Acute risk to fish	100
Chronic risk to fish	10
Acute risk to aquatic invertebrates	100
Chronic risk to aquatic invertebrates	10
Risk to sediment dwelling invertebrates	10
Risk to algae	10
Risk to aquatic plants	10

PPP ecotoxicity and risk assessment

Regulation 546/2011: no authorisation shall be granted if:

- birds and other non-target terrestrial vertebrates
  - acute and short-term TER < 10 (using LD50) or long-term TER < 5
  - secondary poisoning from food (fish, earthworms)
- aquatic organisms
  - fish and Daphnia TER < 100 for acute exposure and < 10 for long-term exposure
  - algal growth inhibition TER < 10
  - BCF > 1000 for readily biodegradable or > 100 for not readily biodegradable AS
- bees
  - hazard quotient for oral or contact exposure > 50
- beneficial arthropods other than honeybees
  - > 30 % of test organisms affected in lethal or sublethal laboratory tests of max application rate
- earthworms
  - long-term TER < 5
- soil microorganisms
  - nitrogen or carbon mineralisation affected > 25 % after 100 days

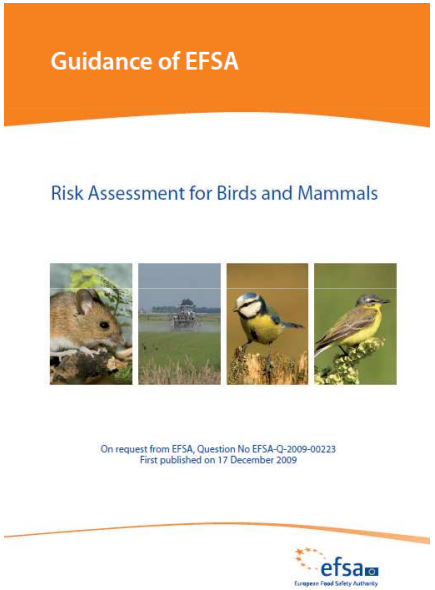
Regulation 546/2011, uniform principles for evaluation and authorisation of PPP
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02011R0546-20180524

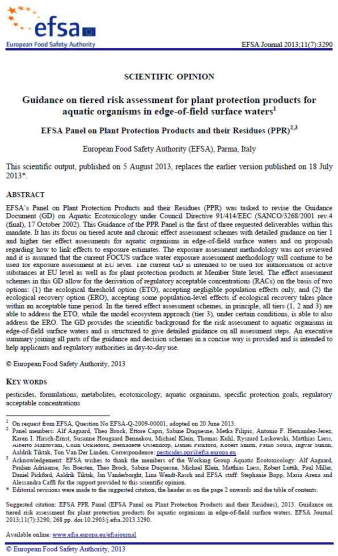
higher tier „unless“:
„unless clearly established through an appropriate risk assessment that under field conditions no unacceptable impact occurs after PPP use in accordance with the proposed conditions of use“

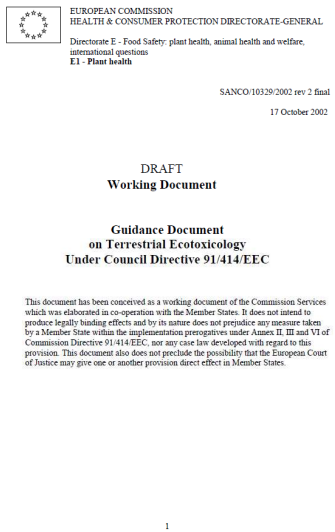
However, in general more detailed decision is performed based on specific guidelines!

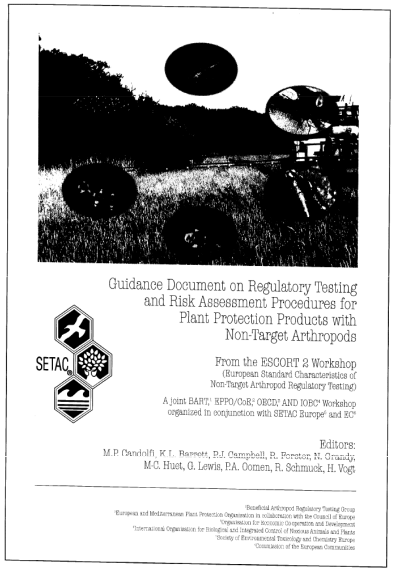
PPP ecotoxicity and risk assessment

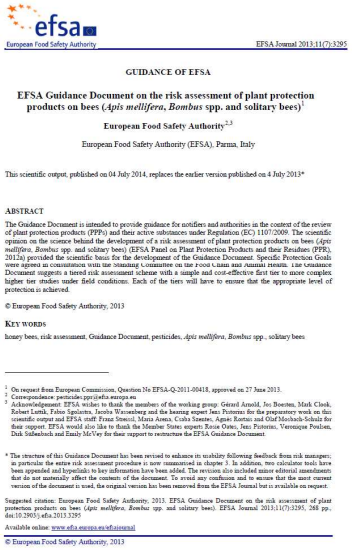
- details for the testing and evaluation (risk assessment) in **guideline documents**
- for ecotox mainly these 4 are of key importance:







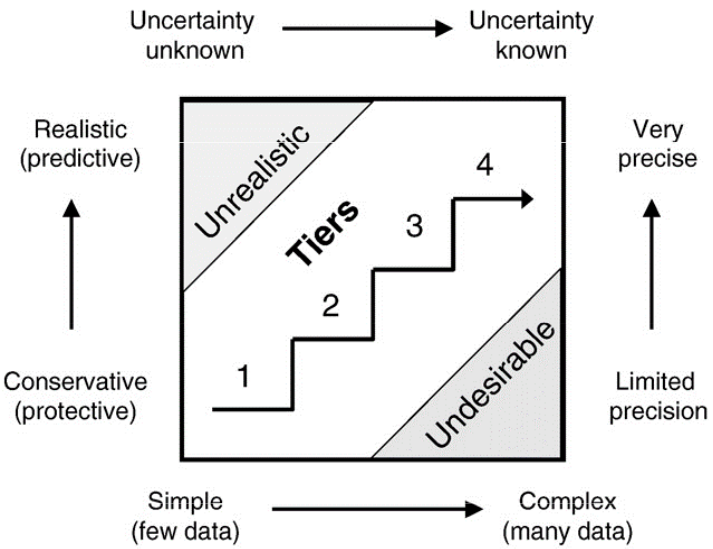




- EFSA (2009): Risk assessment for birds and mammals: EFSA guidance document. EFSA Journal 7(12): 1438. http://www.efsa.europa.eu/en/efsajournal/pub/1438
- EFSA (2013): Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 11(7): 3290. https://www.efsa.europa.eu/en/efsajournal/pub/3290
- European Commission (2002): Guidance document on terrestrial ecotoxicology. Draft Working Document SANCO/10329/2009, rev.2, final. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_ecotox_terrestrial.pdf
- Candolfi (2000): ESCORT2 - Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods.
- EFSA (2013): Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 11(7): 3295. https://www.efsa.europa.eu/en/efsajournal/pub/3295

Tiered approach - general

- this approach saves time, money, laboratory organisms etc.
- it tries to go narrow pathway „linking questions about risks asked by stakeholder to answers that can be provided by researchers“
- its always trade-off between price and accuracy of assessment and these are different for each tier



Posthuma et al. 2008, Science of the Total Environment, 406:503-517

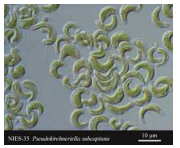
Tiered approach - general

- this approach saves time, money, laboratory organisms etc.

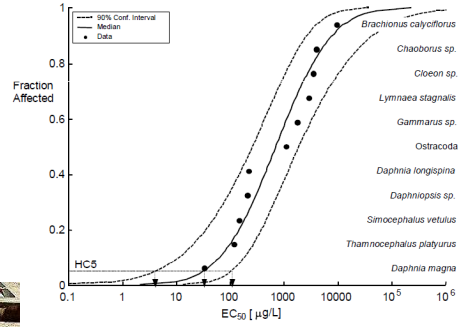
- Tier 1 = starts with default (worst-case) parameters for exposure
  - **= standard ecotox bioassays as we know them**
  - rough assessment, conservative
  - if risk is not acceptable then →
- Tier 2 = refinement, assessment closer to reality (e.g. representative organisms, real conditions, considering protective distances ...), if risks not acceptable then →
- Tier 3, Tier 4 ... e.g. mesocosm or field studies

- based on the results of previous tier, its decided what to do next

Tiered approach - examples





Aquatic organisms

- Tier 1 - based on standard laboratory studies
- Tier 2 - based on additional laboratory studies:
  - Tier 2A – based on geomean/AF approach
  - Tier 2B – based on SSD approach (**Species Sensitivity Distribution**)
  - Tier 2C – based on refined exposure laboratory tests/AF approach
- Tier 3 - based on mesocosm/microcosm studies

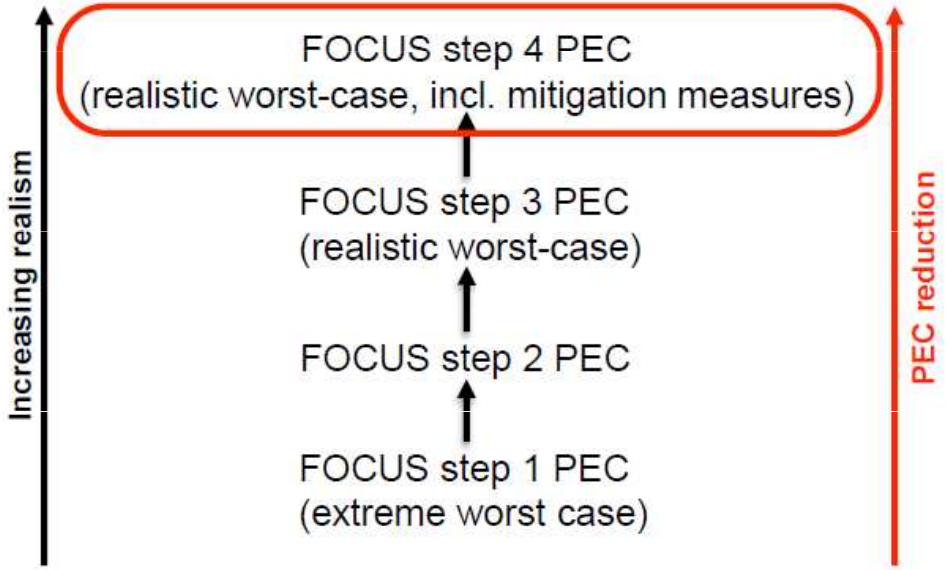
- at each tier, **regulatory acceptable concentrations (RACs)** derived and compared to PEC

EFSA (2013): Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 11(7): 3290. https://www.efsa.europa.eu/en/efsajournal/pub/3290

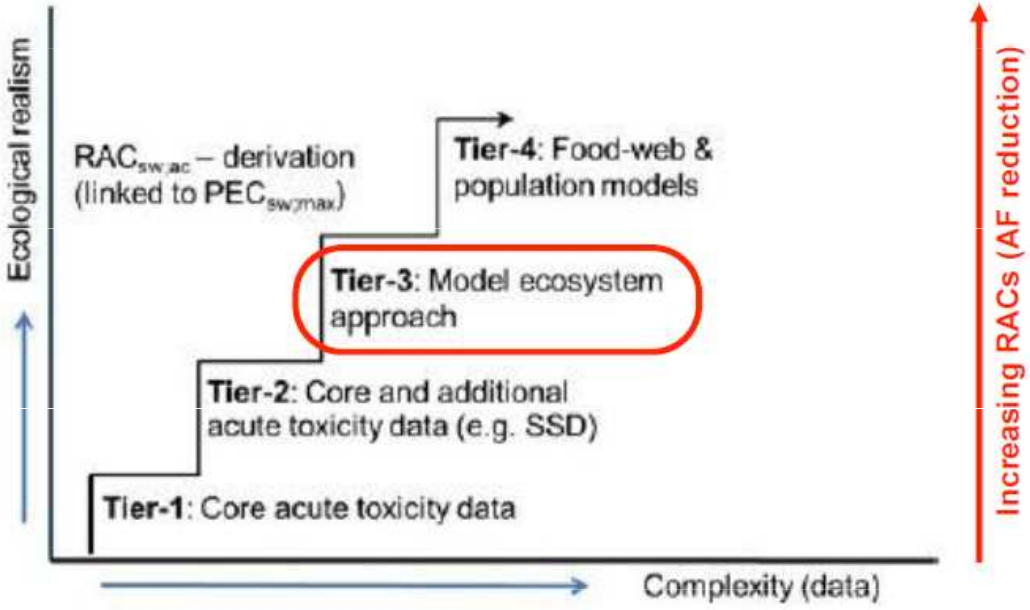
Tiered approach - examples

Aquatic organisms

Exposure assessment



Effect assessment



From: Better Training for Safer Food Initiative workshop, 2019

Tiered approach - examples

Aquatic organisms

Tier I risk assessment

LC_{50} fish: 0.1 µg/l, AF: 100; **tier I RAC (0.001 µg/L)** << step 1 PEC (0.2 µg/L) –> **risk!**

EC_{50} daphnia: 0.11 µg/l, AF: 100; **tier I RAC (0.0011 µg/L)** << step 1 PEC (0.2 µg/L) –> **risk!**

EC_{50} algae: >> 1 mg/L –> **no risk!**

Higher tier risk assessment

Tier 2 (SSD)

Median HC_5 for fish (SSD): 0.072 µg/L, AF: 10; **higher tier RAC (0.0072 µg/L)** > step 4 PEC (0.0049 µg/L) –> **no risk for fish, but still for invertebrates (tier I RAC: 0.001 µg/L)!**

Tier 3 (Mesocosms)

NOEAEC (mesocosm; invertebrates): 0.015 µg/L (clear effects, but recovery within 8 weeks), AF: 3; **higher tier RAC (0.005 µg/L)** > step 4 PEC (0.0049 µg/L) –> **no risk!**

Tiered approach - examples

Birds and mammals

- screening – general model, indicator species
- tier 1 – specific model, generic focal species with different feeding preferences and growth stages
- higher tier – focal species, corrections using measured data (monitoring studies)

Indicator species – used in the screening step, it is **not a real species** but, by virtue of its size and feeding habits is considered to have higher exposure than (i.e. to be protective of) other species that occur in a particular crop at a particular time.
Generic focal species – used in Tier 1assessment, it is **not a real species**, however it is considered to be representative of all those species potentially at risk. Instead of the one single food item approach of the screening step in this assessment a mixed diet is applied when appropriate for the generic focal species. In addition, interception of the spray by the crop is taken into account by calculating the residue level on the several food types for the birds and the mammals.
Focal species – used in higher tier assessment, it is a **real species** that actually occurs in the crop when the pesticide is being used (see section 6.1.3 for identification of focal species.).

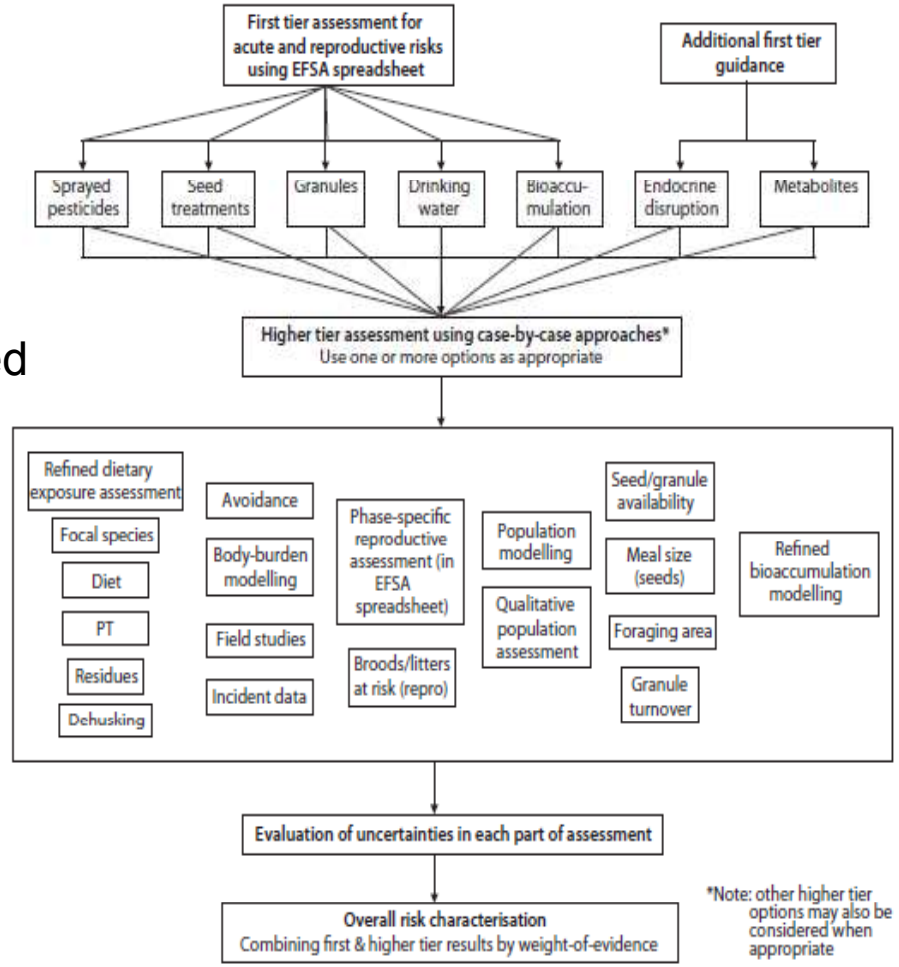
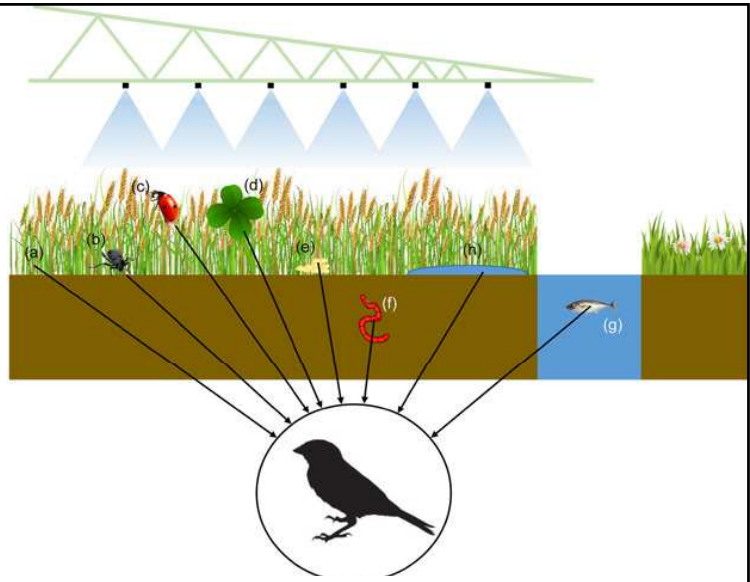


Figure 1. Flowchart for the risk assessment. Please note that for some types of assessment there is an optional screening step.

Tiered approach - examples

Birds and mammals

- **shortcut values** for screening and Tier 1 calculations - describe feeding habits and other ecological needs for indicator and generic focal species



Theoretical dietary exposure routes for birds feeding in crop (e.g., cereals) sprayed with a plant protection product. Most of the plant protection product will be deposited in the treated crop area, but some may enter neighboring water bodies. Exposure to birds in the treated area can therefore occur by feeding on the crop itself (**a**), ground-dwelling (**b**) or foliar-dwelling (**c**) invertebrates, weeds (**d**), or weed seeds (**e**). Birds may also feed on earthworms living in the treated soil (**f**) or fish living in neighboring contaminated surface waters (**g**). Exposure may also occur by drinking from contaminated puddles within the treated crop area (**h**)

Table 6. Acute shortcut values (based on 90th percentile residues) for avian indicator species.

Crop	Indicator species	Shortcut value for acute assessment
Bare soils and hop	Small granivorous bird	24.7
Grassland	Large herbivorous bird	30.5
Bush and cane fruit	Small frugivorous bird	46.3
Orchards and ornamentals/nursery	Small insectivorous bird	46.8
Vineyard	Small omnivorous bird	95.3
Bulbs and onion like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird	158.8
Cotton	Small omnivorous bird	160.3

Table 8. Acute shortcut values (based on 90th percentile residues) for mammalian indicator species.

Crop	Indicator species	Shortcut value for acute assessment
Bare soil	Small granivorous mammal	14.4
Bush and cane fruit	Small herbivorous mammal	81.9
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	118.4
Cotton, fruiting vegetables, grassland, leafy vegetables, legume forage, maize, orchards, ornamentals/nursery, pulses, and vineyard	Small herbivorous mammal	136.4

Tiered approach - examples

Birds and mammals

- **higher tiers, e.g. field studies**




Purpose:
- Determine PT or PD values for use in refinements
- Determine home ranges and further parameters
- Monitoring of population development
- Evaluate potential adverse effects of PPP on birds

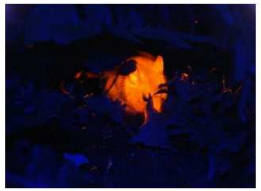
Methods:
- Transect counts
- Scan sampling
- Bird catch - and ringing
- Telemetry (radio-tracking)
- Monitoring of reproduction success
- Carcass search
- Residue analysis in dead animals









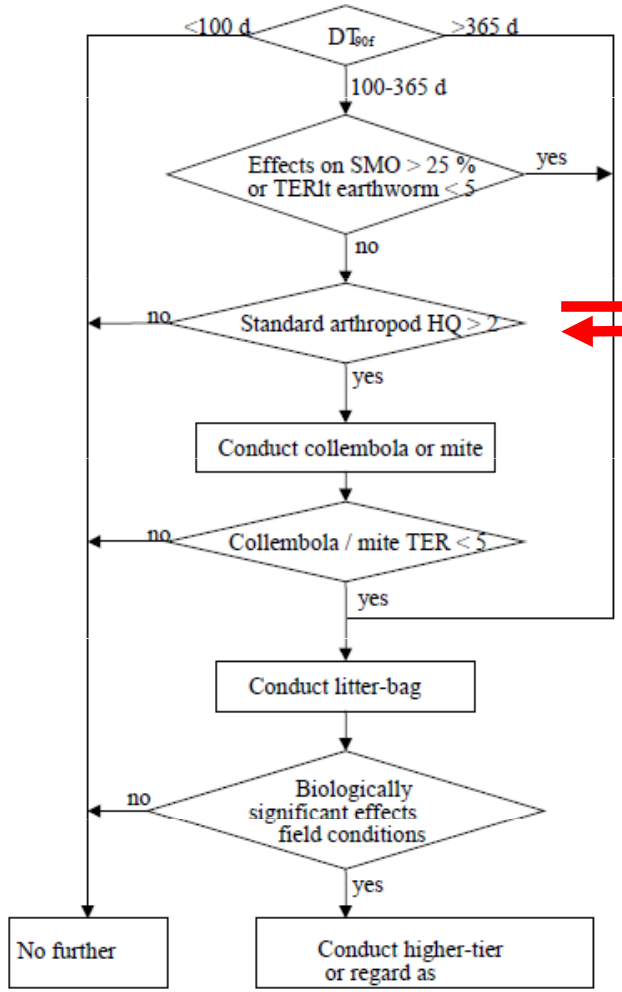


Purpose:
- Determine PT or PD values for use in refinements
- Determine home ranges and further parameters
- Monitoring of population development
- Evaluate potential adverse effects of PPP on mammals

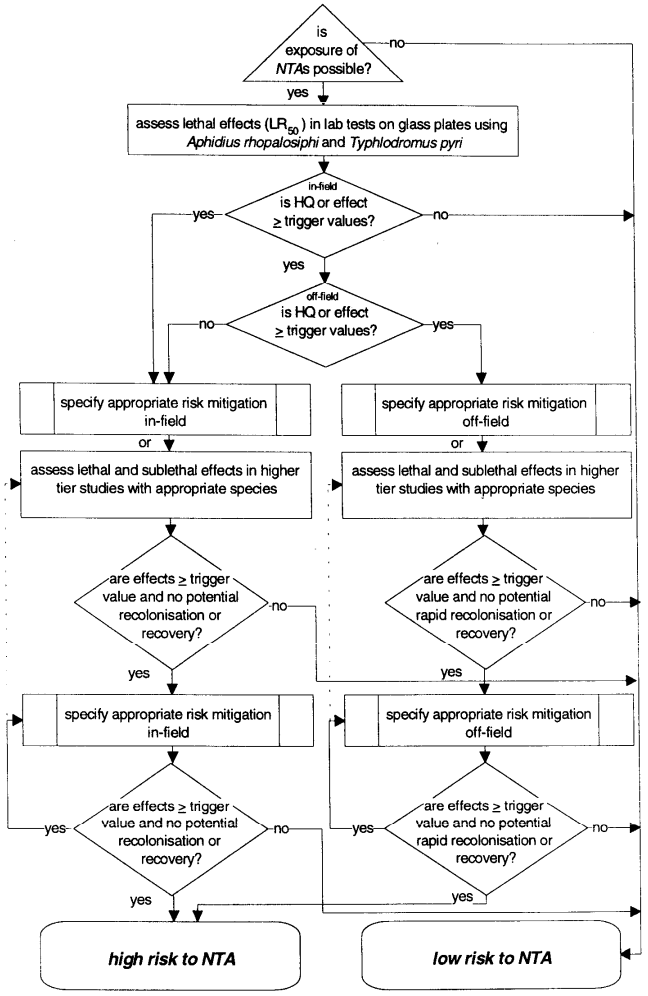
Methods:
- Capture-mark-recapture (CMR), individual markage
- Infrared cameras
- Telemetry (radio-tracking)
- Collection of faeces + analysis of food composition
- Population monitoring
- Carcass search
- Residue analysis in dead animals


Tiered approach - examples

Soil organisms



Non-target arthropods (NTA)

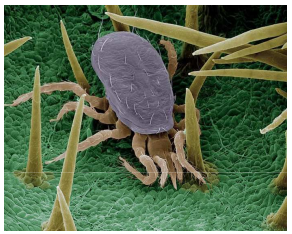


- European Commission (2002): Guidance document on terrestrial ecotoxicology. Draft Working Document SANCO/10329/2009, rev.2, final. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_ecotox_terrestrial.pdf
- Candolfi (2000): ESCORT2 - Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods.

Simplified example of evaluation

Non-target arthropods (NTA)

- **in-field and off-field assessment**



TIER 1

- risk assessment based on two indicator species:
  - Typhlodromus pyri
  - Aphidius rhopalosiphi
- standard toxicity tests to determine LR50 in g a.s. / ha based on mortality
- example results for PPP „Pest-Killer“
  - tested at 1.25, 2.5, 5, 10, 20, 40, 80 g a.s./ha
  - LR50 for both species = 2.5 g a.s./ha
  - all in acreditted labs with GLP

- European Commission (2002): Guidance document on terrestrial ecotoxicology. Draft Working Document SANCO/10329/2009, rev.2, final. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_ecotox_terrestrial.pdf
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Simplified example of evaluation

Non-target arthropods (NTA)

$$\mathbf{HQ} = \frac{\textbf{application rate in g a.s./ha}}{\textbf{LR50 in g a.s./ha}}$$

TIER 1 (cont.)

- now to determine hazards, compare exposure to effect in hazard quotient (HQ) – exposure (application rate) and LR50 must not differ in units
- if resulting HQ ≥ 2, there is potential hazard to non-target arthropods

Single application in g a.s. / ha

Ratio between rate after max. recommended no. of applications and the initial rate after a single application

$$\textbf{In-field HQ} = \frac{\text{Application rate x MAF}}{\text{LR50}}$$

LR50 expressed in g a.s. / ha

= % drift/100 (90th percentile)

For 2-D exposure (= glass plate) this = 10

To account for uncertainty between species this = 10

$$\textbf{Off-field HQ} = \frac{\text{Application rate x MAF x (drift / veg distribution factor) x correction factor}}{\text{LR50}}$$

- European Commission (2002): Guidance document on terrestrial ecotoxicology. Draft Working Document SANCO/10329/2009, rev.2, final. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_ecotox_terrestrial.pdf
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Simplified example of evaluation

Non-target arthropods (NTA)

TIER 1 (cont.)

- **application details:**
  - application rate = 56 g a.s./ha
  - No. of treatments = 3 per crop
  - LR50 = 2.5 g a.s. / ha
- assumptions:
  - Drift value – 2.01
  - MAF – 2.3
  - Application to field crops at tier 1 use the shortest distance – 1 m (3m for orchards, vines etc)

Basic drift values for 3 applications

Distance [m]	Field crops	Fruit crops early	Fruit crops late	Grapevine early	Grapevine late	Hops	Vegetables, Ornamentals, Small fruits Height < 50 cm	Vegetables, Ornamentals, Small fruits Height > 50 cm
1	2.01						2.01	
3		23.96	11.01	2.49	6.90	15.93		6.90
5	0.41	15.79	6.04	1.04	3.07	8.57	0.41	3.07
10	0.20	8.96	2.67	0.32	1.02	3.70	0.20	1.02
15	0.14	5.23	1.39	0.16	0.54	2.26	0.14	0.54
20	0.10	2.36	0.80	0.10	0.34	1.05	0.10	0.34
30	0.07	0.77	0.36	0.05	0.18	0.34	0.07	0.18
40	0.05	0.35	0.21	0.03	0.11	0.15	0.05	0.11
50	0.04	0.19	0.13	0.02	0.08	0.08	0.04	0.08
75	0.03	0.06	0.06	0.01	0.04	0.03	0.03	0.04
100	0.021	0.03	0.03	0.006	0.03	0.01	0.021	0.03
125	0.017	0.015	0.022	0.004	0.02	0.007	0.017	0.02
150	0.014	0.009	0.016	0.003	0.014	0.004	0.014	0.014
175	0.012	0.006	0.012	0.002	0.011	0.003	0.012	0.011
200	0.010	0.004	0.009	0.002	0.009	0.002	0.010	0.009
225	0.009	0.003	0.007	0.002	0.007	0.001	0.009	0.007
250	0.008	0.002	0.006	0.001	0.006	0.001	0.008	0.006

Ground sediment in % of the application rate (77th percentiles)

In-field effects: $\frac{56 \text{ g a.s./ha} \times 2.3}{2.5 \text{ g a.s./ha}}$ **HQ = 51.52**

Off-field effects: $\frac{56 \text{ g a.s./ha} \times 2.3 \times (2.01/100) \times 10}{2.5 \text{ g a.s./ha}}$ **HQ = 10.03**

- **in-field risk is not acceptable (HQ ≥ 2), off-field is also not acceptable (HQ ≥ 2)**

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- European Commission (2002): Guidance document on terrestrial ecotoxicology. Draft Working Document SANCO/10329/2009, rev.2, final. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_ecotox_terrestrial.pdf
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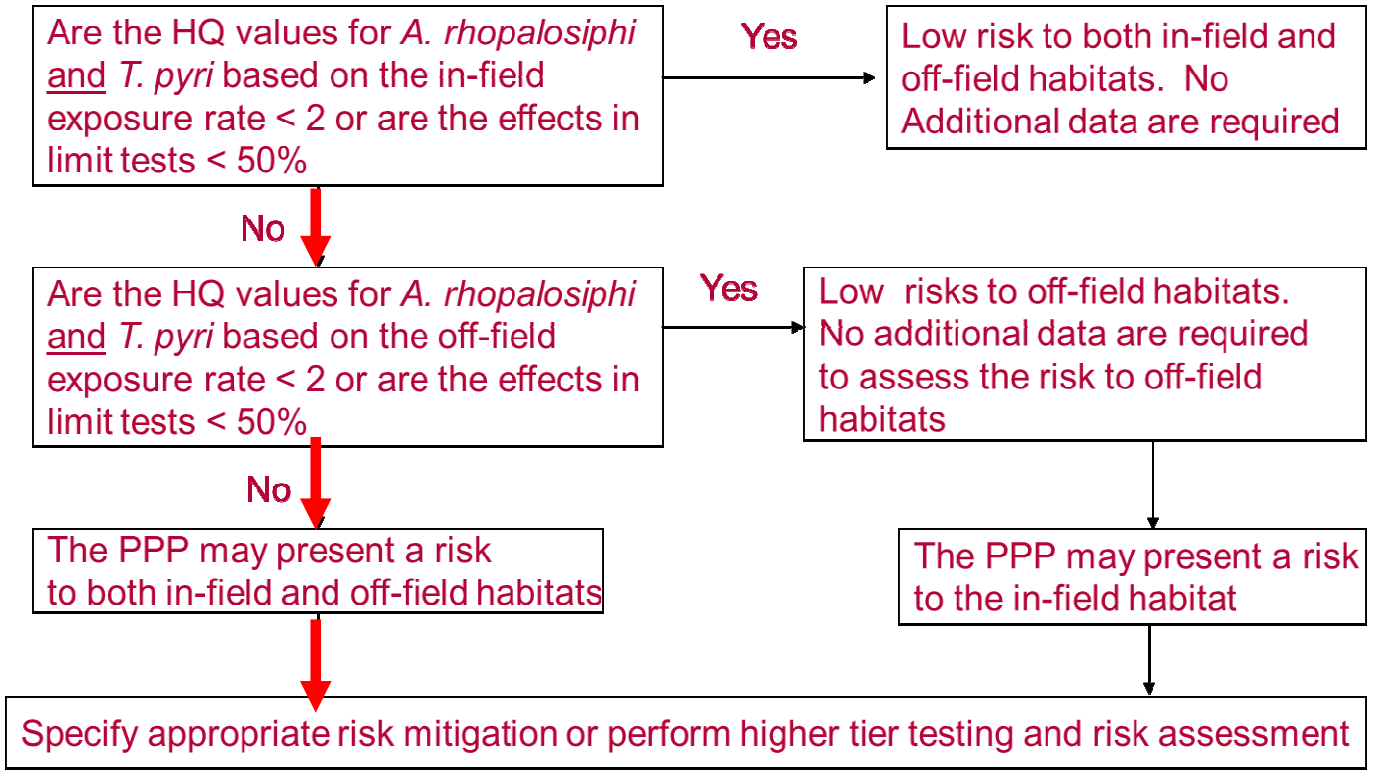
Simplified example of evaluation

Non-target arthropods (NTA)

TIER 1 (cont.)



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Simplified example of evaluation

Simplified example of evaluation: non-target arthropods (NTA)

TIER 2

- if HQ ≥ 2 for in-field risk assessment, test affected indicator species + 1 additional species
- if HQ ≥ 2 for off-field risk assessment, test affected species + 2 additional species
- preferred species: *Orius laevigatus, Chrysoperla carnea, Coccinella septempunctata, Aleochara bilineata, Poecilus cupreus*
-





- **extended laboratory studies; aged residue studies; semi-field studies; field studies**

- European Commission (2002): Guidance document on terrestrial ecotoxicology. Draft Working Document SANCO/10329/2009, rev.2, final. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_ecotox_terrestrial.pdf
- Candolfi (2000): ESCORT2 - Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods.

Simplified example of evaluation

Non-target arthropods (NTA)

TIER 2

- **HQ approach with criteria of ≥ 2 is not applied !**
- predicted exposure rates are calculated based on similar equations as in Tier 1
- compared directly to toxicity endpoints LR50 or ER50 (the lower one should be used)
- **i.e. trigger is less than 50% negative effects**

- European Commission (2002): Guidance document on terrestrial ecotoxicology. Draft Working Document SANCO/10329/2009, rev.2, final. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_ecotox_terrestrial.pdf
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Simplified example of evaluation

Soil organisms

Exposure estimation - PEC_{soil}

- percentage of applied spray volume reaching soil depending on interception (e.g. 50%)
- even distribution in the top 5 cm of soil
- soil density of 1.5 g/cm^3
- calculate PEC_{soil} in mg a.s. / kg soil

- example results for PPP „Pest-Killer“
- application dose is 150 g a.s. / ha
- 1 ha of 5 cm soil corresponds to 500,000,000 cm^3 which is 750,000 kg soil
- **PEC_{soil} is 0.2 mg a.s. / ha**

- European Commission (2002): Guidance document on terrestrial ecotoxicology. Draft Working Document SANCO/10329/2009, rev.2, final. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_ecotox_terrestrial.pdf
- Candolfi (2000): ESCORT2 - Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods.

Simplified example of evaluation

Soil organisms

TIER 1

- reproduction effects on earthworms - NOEC are derived
- TER > 5 OK, TER < 5 further studies needed

- example results for PPP „Pest-Killer“
  - tested at 0.02, 0.04, 0.08, 0.16, 0.32, 0.64 mg a.s. / kg
  - NOEC was 0.32 mg a.s. / kg

- **TER = NOEC/PEC_{soil} = 0.32 / 0.2 = 1.6 (< 5, not OK and further studies are needed)**

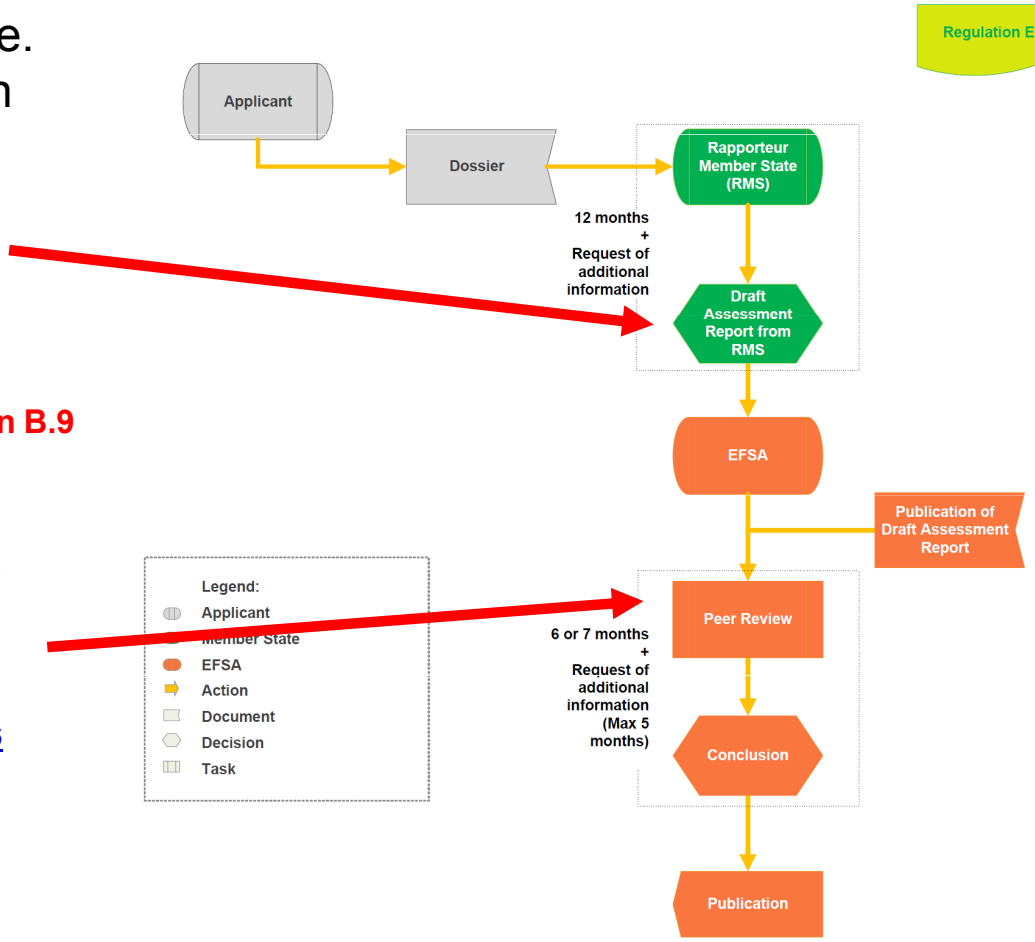
- European Commission (2002): Guidance document on terrestrial ecotoxicology. Draft Working Document SANCO/10329/2009, rev.2, final. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_ecotox_terrestrial.pdf
- Candolfi (2000): ESCORT2 - Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods.

PPP assessment – results available

ecotoxicological data and risk assessment (i.e. data on tox/ecotox are combined with data on exposure and env. fate) are available:

- in Draft Assessment Reports or Renewal Assessment Reports (DAR / RAR)
  http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?5
  (generally: https://www.efsa.europa.eu/en/calls/consultations)
  Part A: Summary of each section; List of Endpoints
  Part B: Detailed evaluation for each area; **Ecotox in section B.9**
  Part C: Confidential information

- in EFSA Conclusions, Peer Reviews and Peer Review Reports; example of Epoxiconazole:
  https://www.efsa.europa.eu/en/efsajournal/pub/rn-138
  https://www.efsa.europa.eu/en/efsajournal/pub/4123
  http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?16



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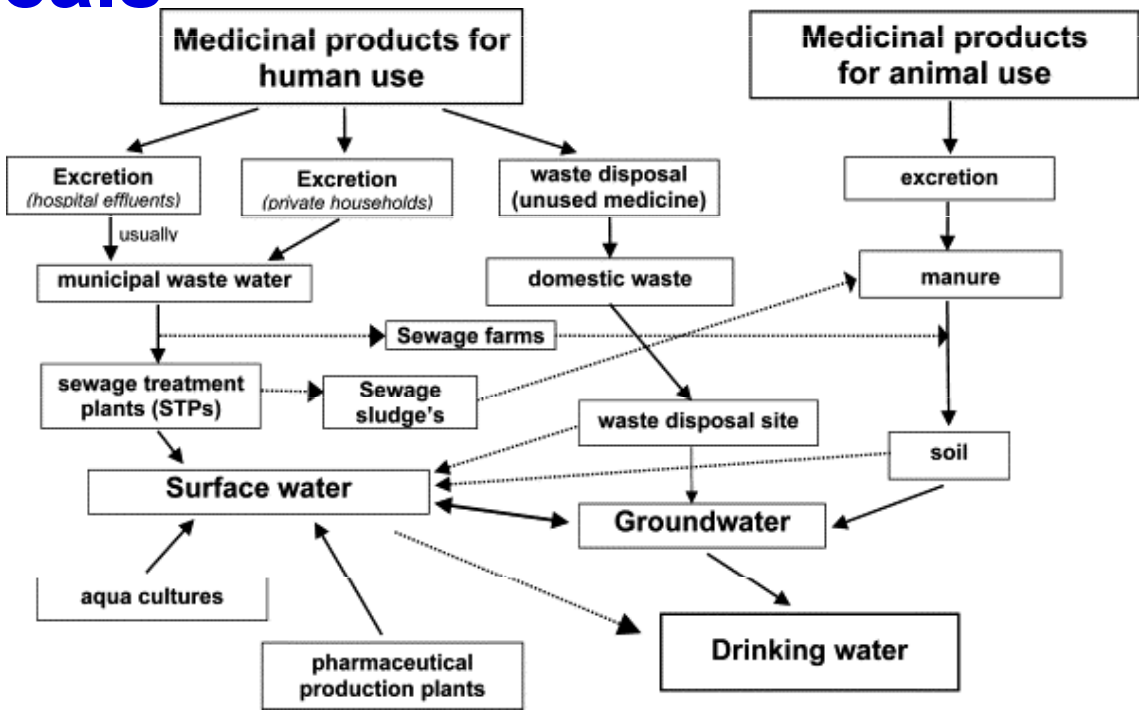
Example 4
Veterinary pharmaceuticals – in EU

Veterinary pharmaceuticals

- **growing problem** – increasing release of bioactive substances to environment



- veterinary medicines - more attention is logical
  - huge consumption
  - their entry into the environment (water and soil) is more possible (use of agricultural waste on soil)
  - killing beneficial soil organisms or development of antibiotic resitance

Antibiotic consumption in major countries/regions

Country/region	Year	Total(tons)	Human(%)	Animal(%)
China	2013	162,000	48	52
USA	2011/2012	1,7900	18	82
EU	2012	11,382	30	70

Veterinary pharmaceuticals

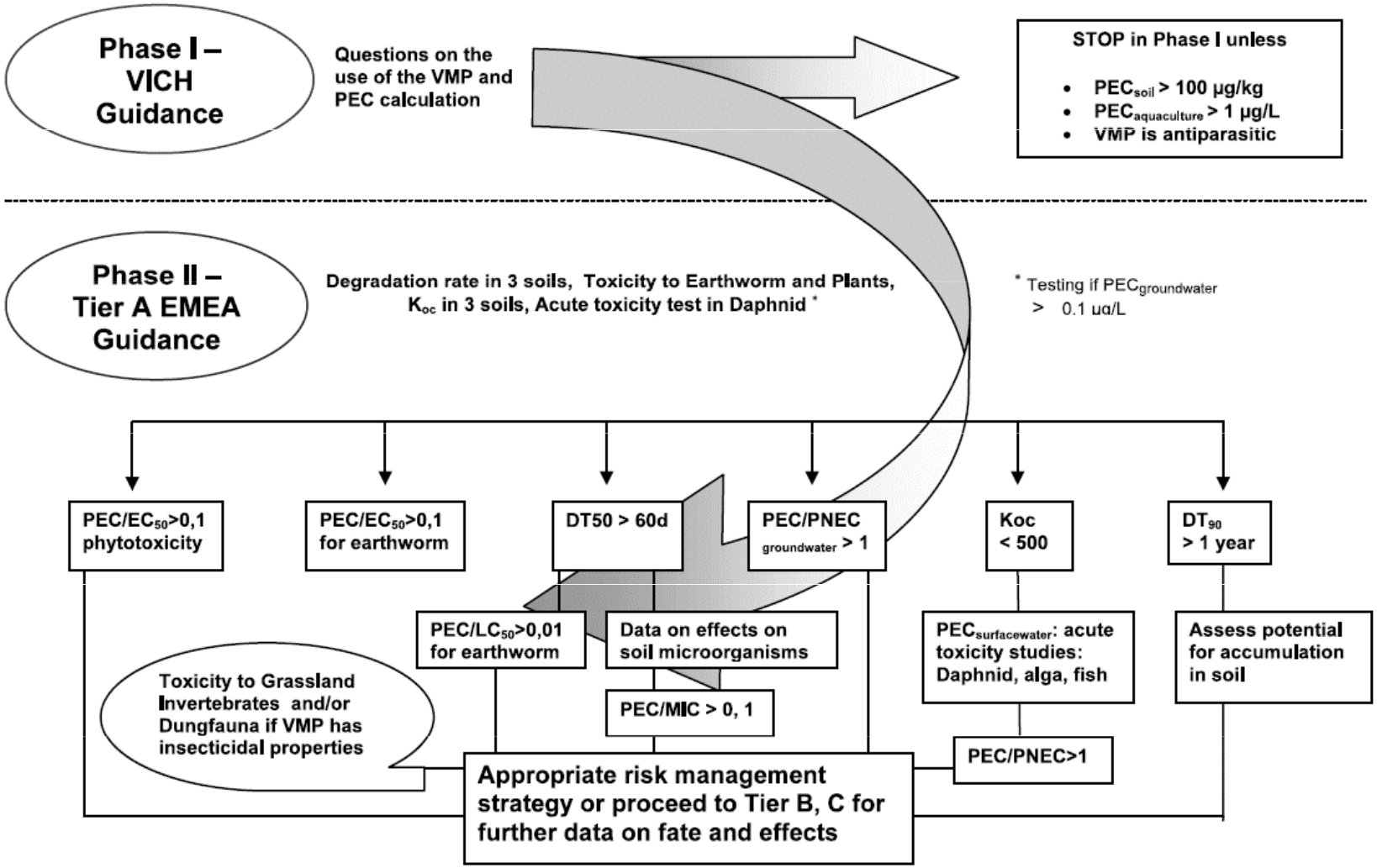
- Regulation 726/2004, authorisation and supervision of medicinal products for human and veterinary use
  https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02004R0726-20190330
- Directive 2001/83/EC, medicinal products for human use
  https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02001L0083-20190726
- Directive 2001/82/EC, veterinary medicinal products
  https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02001L0082-20090807

- **pharmacovigilance** – careful assessment of all undesired impacts
- **European Medicines Agency (EMA)** https://www.ema.europa.eu/en
- part of the registration proces is also **Environmental Risk Assessment**
- for veterinary - detailed evaluation procedure in EMA CVMP (Committee for Medicinal Products for Veterinary Use)
  https://www.ema.europa.eu/en/committees/committee-medicinal-products-veterinary-use-cvmp
- **guidelines including assessment of environmental fate, ecotoxicity and risks**
  https://www.ema.europa.eu/en/veterinary-regulatory/marketing-authorisation/environmental-risk-assessment-veterinary-medicines

Veterinary pharmaceuticals

- **guidelines including assessment of environmental fate, ecotoxicity and risks**
  https://www.ema.europa.eu/en/veterinary-regulatory/marketing-authorisation/environmental-risk-assessment-veterinary-medicines
- VICH GL6 Environmental impact assessment (EIAS) for veterinary medicinal products - Phase I
- VICH GL38 Environmental impact assessments for veterinary medicinal products - Phase II

Veterinary pharmaceuticals



Koschorreck, J., Koch, C., Rönnefahrt, I., 2002. Environmental risk assessment of veterinary medicinal products in the EU—a regulatory perspective. Toxicology Letters 131, 117–124.. doi:10.1016/s0378-4274(02)00047-4