PROSPECTIVE AND RETROSPECTIVE

ENVIRONMENTAL RISK ASSESSMENT OF MIXTURES

MOVING FROM RESEARCH TO REGULATION

# Mixture toxicity considerations for Plant Protection Products - Ecotoxicology

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- Required ecotoxicology testing
  - Terrestrial organisms (birds, mammals, NTAs, bees, NTPs, soil macro- and micro-organisms)
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- Considerations
- Conclusions

# Plant Protection Products
## - a special case

- Designed to have a specific biological effect
- Introduced intentionally into the environment
- Therefore, extensive data requirements, both for ecotoxicology and environmental fate
- Well characterized chemicals
- Most strictly regulated chemicals

# Relevance of mixtures

- All formulated PPPs are mixtures
  - one a.i. plus other substances (water, surfactants, bactericides, adjuvants, UV stabilizers etc.)
  - multiple a.i.‘s plus other substances (water, surfactants, bactericides, adjuvants, UV stabilizers etc.)
- Tank mixes (combination of formulations)
- Sequential spraying of different formulations
- Post registration monitoring data will likely detect a combination of pharmaceuticals, PAHs, metals, nutrients, PPPs etc.

**-> This is only chemical stress; what about other stressors, e.g. temperature, light, noise, ploughing, invasive species?**

# Relevance of mixtures

This presentation focusses on pre-registration data and **prospective** risk assessment, not on retro-spective (e.g. monitoring) data, because the latter is not specific to PPPs

**Defined mixtures** vs. undefined mixtures

-> presentations of Theo Brock (WFD) and Paul Price (monitoring data)

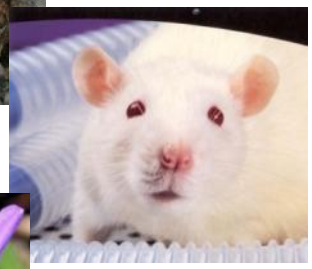
# What is the benefit of using mixtures?

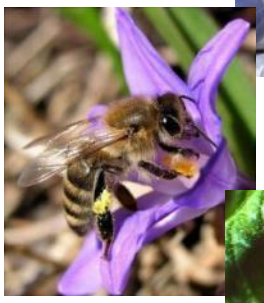
- The non-a.i. constituents of a formulation are tailored to stabilize and deliver the a.i. and its activity to the target, so less of the a.i. is required
- In case of multiple a.i.‘s:
  - reduce resistance (combination of different MoA)
  - broader spectrum of pest control
  - lower amount of individual a.i.‘s required
  - less fuel; single run for farmer

# Required testing

- For many organism groups data are required on both the product (i.e. mixture) and the a.i., enabling comparisons
- In case the product is significantly more toxic than expected from the a.i., further data may be required and/or the risk assessment is based on the formulation endpoints

# Effects Assessment - Ecotoxicology
# Data Requirements for PPPs





## Terrestrial organisms

- Birds and mammals
- Bees
- Non-target arthropods
- Earthworms & other soil macrofauna
- Soil micro-organisms
- Non-target plants

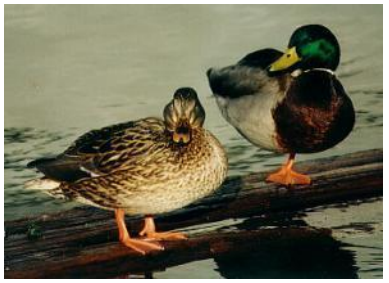
## Aquatic organisms

- Fish
- Invertebrates
- Algae and macrophytes

# Terrestrial organisms

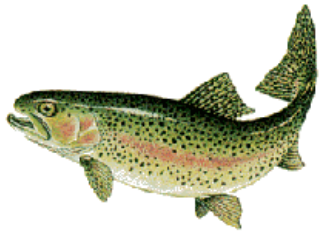
- Non-target arthropods and non-target plants: only product testing -> mixture effects included
  - direct exposure
- Honeybees: at tier 1 both a.i. and product. Higher tier only product testing -> mixture effects included
  - direct exposure
- Soil organisms: at tier 1 both a.i. and product. Higher tier only product testing -> mixture effects included
  - indirect exposure

# Birds and mammals

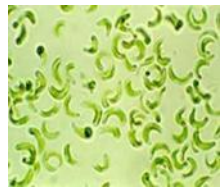


- Acute rat studies both a.i. and product
- Normally no acute bird testing for products (for animal welfare reasons)
- Long-term studies only with a.i., since the formulation does not persist in the environment (plant, water, soil)
  - (In)direct exposure
- New Guidance provides step wise approach for combined risk assessment
  - Consideration of MoA for chronic effects

# Aquatic organisms



- In chronic studies on algae (and *Lemna*) both a.i. and product
- In short term studies on fish and *Daphnia* both a.i. and product
- If the product is significantly more toxic than expected from the a.i. (factor of 10), chronic product data may be required
- The relevance of chronic formulation data is questionable (Sanco, 2002; Creton et al., 2010) since the formulation does not persist in the aquatic environment
  - Indirect exposure



# Comparing a.i. and formulation

- the toxicity of the formulation (= mixture) is often dominated by a single a.i.
- If a.i. content in a formulation is low, deviation more likely
- If non-target species is closely related to target species (e.g. *Lemna* – herbicides) prediction based on a.i. toxicity good
  - **These species typically drive the risk assessment**

# Risk assessment: Independent action (IA) and concentration addition (CA)

- In the risk assessment there is an implicit consideration of IA by assuming toxicity stems from one a.i.
- When one a.i. dominates the toxicity of the mixture, IA is hardly distinguishable from CA
- Short-term endpoints more likely to show CA, while in chronic tests IA more likely
- Overall, CA seems appropriate, somewhat conservative (Kortenkamp et al., 2009; Verbruggen and van den Brink, 2010)
- Mixture toxicity ranges roughly from 0.3 to 3.0 toxic units
- Synergism is rare (antagonism is ignored in risk assessment)

-> **These conclusions are mainly derived from laboratory data**

# Extrapolation to the field

**Are interactions observed in the laboratory at near threshold (NOEC or $EC_{50}$) levels also occurring in the field?**

- Thus, for fish or daphnids, at $1/10^{th}$ of the NOEC or $1/100^{th}$ of the $EC_{50}$ for individual a.i.‘s
  - Not likely to be equitoxic mixtures
  - Exposure peaks of a.i.‘s only overlap for drift entry

# Extrapolation to the field - consistency

- The nature of the interaction depends on the exposure time (Baas, 2010)
- The nature of the interaction depends on the chosen effect level and on the chosen endpoint (Cedergreen and Streibig, 2005)
- Reproducibility of mixture result *in vivo* is poor (Cedergreen et al., 2007; Munkegaard et al., 2008)
  - consider variability in endpoints (e.g. *Daphnia* $EC_{50}$ alpha-cypermethrin 0.16 – 0.62 µg/L, Norgaard and Cedergreen, 2010)

# Synergism I

- Pyrethroids and EBI-fungicides are a known case of synergism
  - Mechanism well understood: EBI‘s inhibit metabolic (P450) breakdown of pyrethroids (Wilkinson et al., 1974)
- Mainly demonstrated in laboratory studies
  - Honeybees: Pilling (1992), Colin and Belzunces (1992)
  - Daphnia: Norgaard and Cedergreen (2010)

# in the field?

- Field studies of EBI-pyrethroid mixtures found no synergy
  - Honeybees: Lefebvre and Bassand (1999)
  - Aquatic invertebrates: Lindner et al. (2010)

**due to concerns over bees, tankmixes of pyrethroids and EBI fungicides are under restriction**

# Synergism II

- Enhanced toxicity to birds of malathion in the presence of prochloraz in the lab (Johnston et al., 1989)
  - Not in the field (Johnston et al., 1996)
- Enhanced toxicity to bees of thiacloprid in the presence of tebuconazole/prochloraz in the lab (Schmuck et al., 2003)
  - Not in the field (Schmuck et al., 2003)

# in the field?

- Sequential spraying (crop approach) in semi-field studies in Wageningen (NL) indicated no synergism; effects were determined by the most toxic a.i. (Verbruggen and van den Brink, 2010)

# Conclusions

- Potential for mixture toxicity would be picked up by current testing requirements for a.i. and formulation
  - may trigger further testing
  - Focus on species that drive the risk assessment
- Current risk assessment practise with its assessment factors appears to cover mixture effects
  - typically between 0.3 and 3.0 TU based on CA
- Considering normal experimental variation in test outcome with single chemicals it should be considered if mixture toxicity is a relevant issue for PPPs.

**Thank you for your attention**

Any questions?


Early environmental risk assessors.