

**CLASSIFICATION OF  
DEVELOPMENTAL TOXIC  
PESTICIDES  
AND NEGLIGIBLE EXPOSURE**

*Perspective of the regulator*

**Alberto Mantovani**

**Dept. Food safety, Nutrition, and Veterinary Public Health**

**Istituto Superiore di Sanità, Roma, Italy,**

**[alberto.mantovani@iss.it](mailto:alberto.mantovani@iss.it)**

## NEGLIGIBLE EXPOSURE

An active substance, safener or synergist shall only be approved, if not classified *toxic for reproduction category 1A or 1B*, **or** not considered to have *endocrine disrupting effects that may cause adverse effects*

**unless**

Exposure in humans **is negligible:**

*(non-dietary)*

- product is used in closed systems or
- in other conditions excluding contact with humans

and where *(dietary)*

- residues on food and feed do not exceed the *default value*

**(0.01 mg/kg) set in accordance Regulation (EC) No 396/2005**

## ***“NEGLIGIBLE” ?***

**‘Negligible’ is not equal to zero (the “myth” of ZERO exposure)**

***Oxford English Dictionary =***

**"so small or unimportant as to be not worth considering; insignificant".**

***Negligible implies risk management decisions which get their basis from risk assessment***

**‘negligible’ can be considered a level so small that it does not appreciably add to the risk and can safely be ignored**

***(Draft Technical Guidance on Negligible Exposure under Realistic Conditions of Use - European Commission, 2015)***

## **EFSA assesses Negligible Exposure (2017)**

**Active substance Pymetrozine pyridine azomethine, insecticide  
Repro Cat 2 (malformations of the pubis in rats and rabbits with low  
maternal toxicity, NOAELs 30 and 10 mg/kg bw in rats, and rabbits  
also impaired sperm production in adult rodents,  
possible enzyme effect – steroidogenesis ??)**

*Dietary* - requested uses in potato and oilseed rape:  
*individual* residues of pymetrozine and metabolites unlikely to  
exceed 0.01 mg/kg

*But* incomplete toxicological characterization of the pertinent plant  
metabolites

*Non-dietary* – recommended **a margin of exposure > 1000**  
(NOAEL for relevant effect to actual/estimated exposure of  
*operators/workers/bystanders/residents (EFSA 2014)*  
acute exposure of operators without risk mitigation measure  
= MoE < 1000

*Therefore “NEGLIGIBLE” exposure can be challenged*

by regulatory developments that can highlight new aspects of *Hazard identification/Characterization*, like

- endocrine-related developmental toxicity, as flagged by the inclusion of **ano-genital distance (AGD)** in the revised OECD TG 414 (2018)
- **developmental neurotoxicity**

By new approaches to define *toxicologically relevant exposures* such as

- **identification of toxicologically relevant residues**
- **cumulative assessment groupings**

## Some comments on AGD

- *Newly introduced regulatory* endpoint in prenatal developmental toxicity (new OECD 414, 2018)
- the most relevant is **AGD relative to body weight**
- **Androgen (and androgen/estrogen) dependent**: highest sensitivity in fetal masculinization programming window (8-14 wk gestation in humans)
- *corroborated by human studies*: rather than an adverse effect per se is a **lifelong predictor of prenatal androgen disruption**
- A cross-cutting predictor*: beyond AR antagonism-ER agonism, **androgen-related pathways**: e.g., DBP (steroidogenesis through PPAR- $\alpha$  and CoAR), possibly also prolactin (Camargo et al., 2017)
- if applied and interpreted in a robust way, it can implement classification and/or NOAELs (**usually more conservative**)

## **Developmental neurotoxicity (OECD TG 426 and beyond)**

- Main issue for pesticides, *not consistently tackled in the EU*
- TG 426 or DNT cohort in OECD TG 443 (EOGRT) can be *triggered* by standard adult and/or reproductive toxicity tests
- However, an accurate appraisal of neurotoxicity and DNT may **significantly impact on safety parameters** (see lowering of ADI/AOEL/ARfD for neonicotinoids, EFSA 2013)

### **OECD-EFSA Workshop report (2017): *tiered strategy***

*In vitro* assays needed in new harmonized testing framework  
in vitro screening and prioritization (for further testing)  
inclusion of DNT in vitro data as part of weight of evidence

- In principle, a more robust and consistent tiered approach to DNT might lead to **increase active substances classified as developmental toxicants**

**And now let's go to**

**New approaches to define  
*Toxicologically Relevant Exposures***

**Which may modify the definition of  
*Negligible***

# The identification of Toxicologically Relevant Residues (EFSA guidance 2016)

- Residues often *do not coincide* with the parent substance
- a number of different compounds resulting from abiotic or biotic (plant) transformation, which
  - can *sum up* with the active substance (comparable toxicity, possibly using relative potency factor )
  - or have **qualitatively** different profile
- first tier of residue assessment is the *genotoxicity* potential
- 2nd tier, other properties of concern: while DevTox is not easily amenable to QSAR, *ReadAcross* methods can be used

There are several cases....

## Toxicologically Relevant Residues (II)

- parent compound has *no DART precedents* and the tested metabolite is *qualitatively similar*,  
*no further testing* to explore DART endpoints
- if the tested metabolite is **considered qualitatively different** from parent compound **either with or without** DART precedents, then *options*:
  - apply an additional safety factor of 10 to the metabolite RD;
  - testing of the metabolite in OECD TG 422
  - direct testing of the DART endpoints (TG 414, 416, 443))
- the parent compound *has DART precedent* and the tested metabolite *is qualitatively similar*, *options*:
  - the same hazard for the metabolite would be assumed, or
  - testing for the DART endpoint of interest

## Two examples from EFSA 2016 (III)

- **Spiroxamine**: Cat 2 DART – cleft palate rats (NOAEL of 30 mg/kg, vs. 10 mg/kg NOAEL used for ArfD)
- Many metabolites
- one group similar to parent compound
- The representative of another group (M03) less potent than parent in repeated dose toxicity: *DART testing is waived* for this group
- A few metabolites of qualitative and quantitative relevance are identified and *require further assessment*.

## Two examples from EFSA 2016 (IIIb)

- **Epoxiconazole (EP):** *Cat 1b DART* – high embryofetotoxicity in rodents and rabbits, steroid synthesis inhibition (DART NOAEL of 20 mg/kg is basis for ArfD)

- Many metabolites: for conjugated metabolites (i.e. glucosides and glucuronides) testing in vitro for enzyme effects will assess **the Relative Potency in comparison to EP**

- Other metabolites (group B) highly structurally similar to EP: no further testing required

- Another group of metabolites (group C) can have significant differences from EP

M06 (expected to be the *most reactive*) should be tested in *OECD 414*

and in vitro for enzyme disruption (possibly in comparison with EP)

## Cumulative exposure (EFSA, 2013)

- Consumer exposure to residues may be *viewed differently* when considering that

More than 20% of fruit/vegetable samples show **residues of multiple active substances** according to EU residue monitoring programmes  
Compounds with **similar effects in the same target organ** may have **additive effects**, *irrespective* of chemical structure and molecular mechanism (“phenotypic effect”)

the new OECD 414 requires the assessment of *thyroid activity in the dam*

(the foetal thyroid depends from maternal thyroid, thus impaired maternal thyroid = prenatal thyroid disruption), thus..

- Cumulative assessment grouping **for thyroid** in (EFSA 2013)

## Cumulative assessment grouping for thyroid (EFSA, 2013)

- cumulative assessment grouping defined by effects occurring at level of *organ* (thyroid follicular cells) or of *system* (hypothalamic-pituitary-thyroid axis) through changes in thyroid hormone levels

Substances affecting thyroid follicular cells, displaying changes in T3/T4 or TSH levels, eliciting follicular cell hypertrophy/hyperplasia or neoplasia, **are allocated in the same group (in total 96 substances).**

The specific effects used to define this group are **apparently interrelated to one another by a chain of events.**

While the precise mechanism of action *is currently unknown* for many substances, and further refinements are expected by increased knowledge

several different mechanisms of action **are expected to contribute to a final deleterious common effect, i.e. decrease in T3/T4 action.**

## *In conclusion*

**New insights in testing may put new substances among those where “negligible exposure” has to be assessed because of their classification as Developmental Toxicants**

**New insights in risk assessment may impact on the definition of negligible exposure**

**Negligible exposure *handle with care***

*Thank You for patient listening!*

